International Journal of Pharmaceutics, 14 (1983) 133–142
Elsevier Biomedical Press

Research Papers

Photo-oxidation of tetracycline—a differential pulse polarographic study

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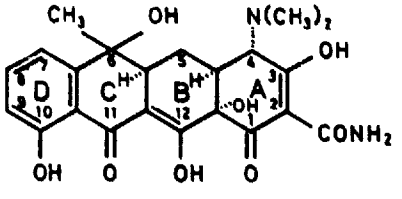
(Received March 24th, 1982)
(Accepted August 19th, 1982)

Summary

Solutions of tetracycline irradiated with UVA light have been analyzed by differential pulse polarography. The polarographic activity of tetracycline results partly from reduction of the 4-dimethylamino group which is removed in the primary photoreaction. Subsequent reactions depend upon the solvent and the presence of oxygen. In aqueous air-saturated solutions at pH 9.0, the end product appears to be a quinone. The existence of free radical intermediates in the photoreaction was confirmed by the addition of acrylamide in surfactant solutions, with observation of the subsequent polymerization.

Introduction

The antibiotic tetracycline (I) may degrade by at least 4 different pathways under various conditions—epimerization, dehydration, hydrolysis and oxidation. Oxidative degradation occurs in alkaline media and is initiated by UVA light, with the formation of a metastable red product (Wiebe and Moore, 1977; Davies et al., 1979). The participation of UVA light suggests that this reaction may be important in



I

initiating the photobiological processes manifest as adverse photosensitivity effects, widely reported for tetracycline (Magnus, 1976).

In this paper we have extended the study of the photo-oxidation of tetracycline, principally using the technique of differential pulse polarography. Tetracycline is polarographically active with a complex reduction pattern believed to be the result of electron transfer processes occurring in ring A of the molecule (Smyth and Smyth, 1978; Smyth et al., 1978). Several of the modern polarographic techniques have been suggested for the analysis of tetracycline, including alternating-current polarography (Caplis et al., 1965; Oliff and Chatten, 1977) and differential pulse polarography (Jochsberger et al., 1979; Cutie et al., 1980). Any oxidation products are expected to be susceptible to reduction at the dropping mercury electrode, thus indicating polarography to be a suitable method for detection of the extent of oxidation of tetracycline.

Materials and methods

Samples of tetracycline base and hydrochloride were kindly supplied by Pfizer Laboratories, Sydney. The absorbance ratio method (McCormick et al., 1957) showed them to be at least 99% pure.

4-Dedimethylaminotetracycline was synthesized according to the method of McCormick et al. (1957). 1,4-Benzoquinone (Fluka) and 1,4-naphthoquinone (Hopkin and Williams) were recrystallized from ethanol. All other chemicals used in polarographic measurements were of analytical grade (Ajax Chemicals, Sydney). All experiments at pH 9.0 were performed in 0.05 M Tris(hydroxymethyl) aminomethane–HCl buffer prepared with doubly distilled water. Stock solutions of tetracycline at pH 9.0 kept in the dark showed no change of polarogram or spectrum after 48 h.

Irradiations of tetracycline solution (5×10^{-5} M) were performed in a cylindrical pyrex glass vessel with a 100 W Hanovia medium-pressure mercury lamp with the apparatus previously described (Wiebe and Moore, 1977). The maximum output of the lamp was at 365 nm and the glass effectively filtered out radiation below 310 nm.

Polarograms were recorded after deoxygenation of the test solution (15 min bubbling with nitrogen) using a PAR Model 364 Polarographic Analyser in the differential pulse mode, at a scan rate of 2 $mV \cdot s^{-1}$. A 3-electrode system was employed, consisting of a dropping mercury electrode with a controlled drop time of 1 s, platinum counter electrode and saturated calomel reference electrode.

Photo-oxidation reaction rates were measured using a polarographic oxygen electrode and stirrer in the pyrex reaction vessel. The oxidizable acceptor, 2,5-dimethylfuran (DMF), was obtained from Aldrich Chemicals, and purified by twice distilling at room temperature under reduced pressure. The reaction vessel was converted into a dilatometer for polymerization rate measurements by fitting a capillary tube in place of the oxygen electrode. Acrylamide monomer (BDH Chemicals) was twice recrystallized from redistilled chloroform.

The surfactants used were cetrimide (BP grade from ICI Australia), cetamocrogol

1000 (BP grade, Prosana Labs, Sydney) and sodium dodecyl sulphate (BDH, U.K., specially pure grade).

The apparatus and procedure for photo-oxidation and photopolymerization have been described previously in greater detail (Moore, 1977). Experiments with surfactants were also as previously described (Moore and Burt, 1981). Fluorescence measurements were made using an Aminco-Bowman Spectrofluorimeter with 150 W Xe lamp, and were uncorrected for variable spectral output.

Results and discussion

Fig. 1 shows polarograms after various times of irradiation with near-UV light of air-saturated solutions (5×10^{-5} M) of tetracycline hydrochloride in pH 9.0 buffer. At this pH, the rate of photo-oxidation is relatively high (Wiebe and Moore, 1977) and the initial polarogram is both less intense and less complex than that at lower pH values. The two waves in Fig. 1 at $t = 0$, designated E_1 at -1.35 V and E_2 at

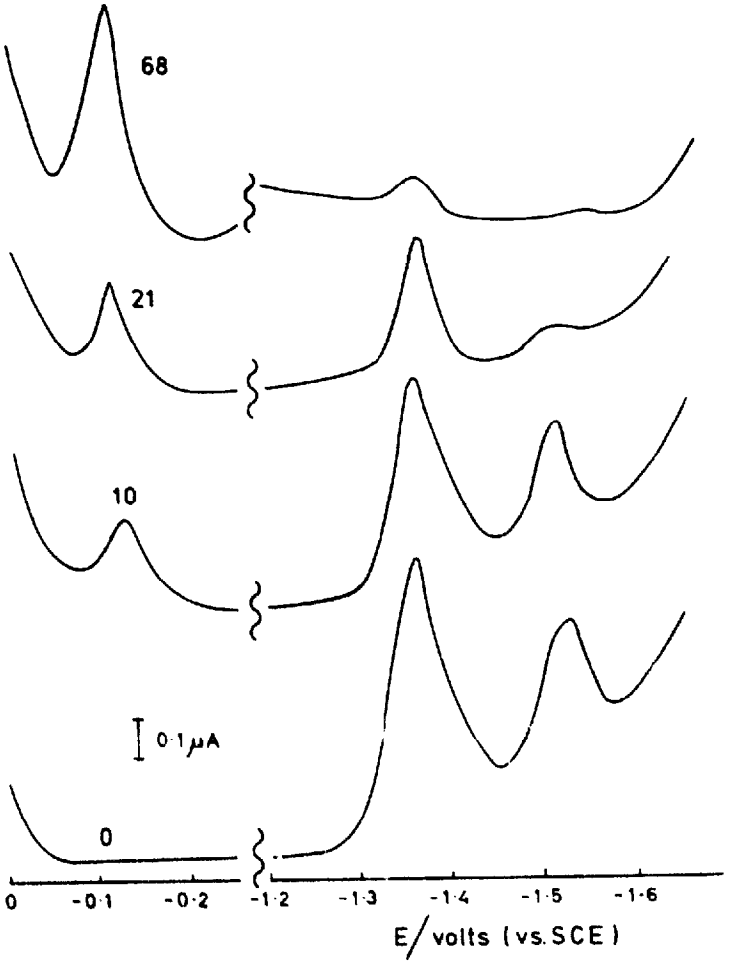


Fig. 1. Differential pulse polarograms recorded from tetracycline (5×10^{-5} M) in air-saturated buffer solutions at pH 9.0 and 30°C irradiated for the times indicated (in min).

– 1.54 V (vs SCE), each appear to be further subdivided at pH 4–6. On the basis of evidence including complexation studies with Ca^{2+} and barbitone at pH 4.7, Smyth et al. (1978) attributed the first wave(s) to reduction of the 4-dimethylamino function, and the second group of waves to the reduction of the keto group at position 1. By a different approach, we are able to confirm their assignment, since irradiation of tetracycline base in methanol results in deamination (Hlavka and Bitha, 1966) and is seen as a loss of the first wave in the polarogram at pH 5.0, while the other waves are only slightly affected. However, at higher pH, the polarogram changes position and shape, and the above assignment would appear to be reversed at pH 9.0, since: (i) 4-dedimethyl-aminotetracycline (V) shows a single wave at

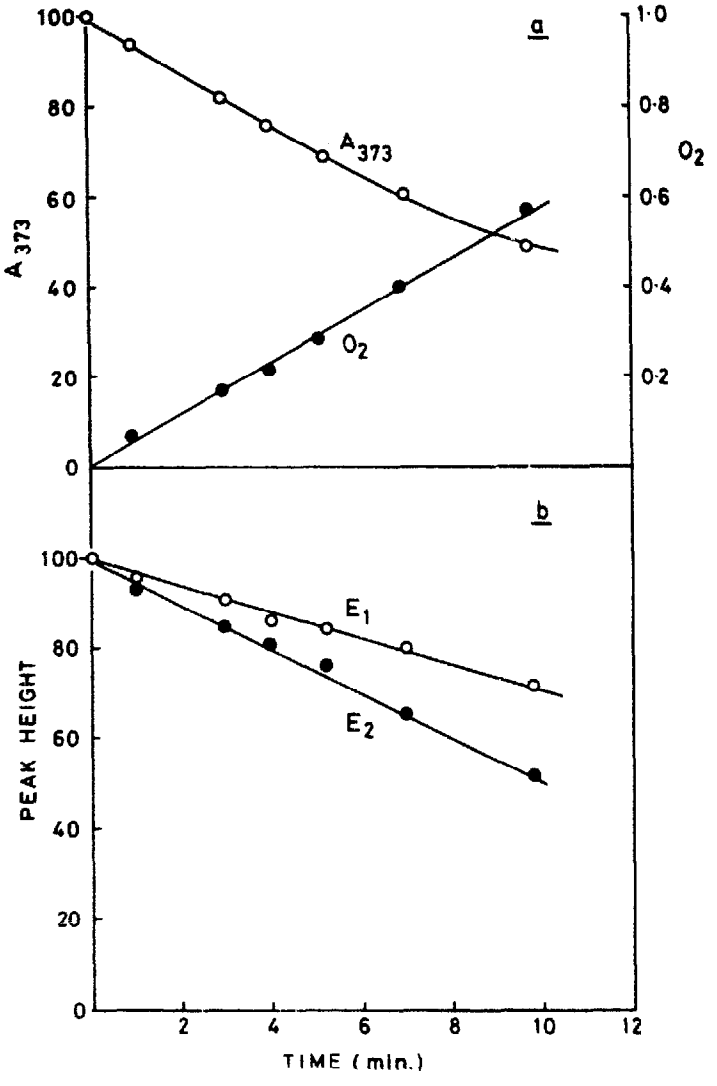


Fig. 2. Relationship between oxygen uptake, absorbance and polarographic changes as a function of irradiation time for 50 μM tetracycline hydrochloride in pH 9.0 buffer solution at 30°C. a: absorbance at 373 nm expressed as % of initial value (left-hand ordinate) and oxygen uptake expressed as moles O_2 consumed per mole of tetracycline (right-hand ordinate). b: peak heights of waves at – 1.35 V (E_1) and – 1.54 V (E_2) expressed as % of initial values in the differential pulse polarogram of tetracycline.

– 1.36 V, corresponding to E_1 of tetracycline; and (ii) E_2 for tetracycline base at pH 9.0 is about 60% of the height observed for tetracycline hydrochloride, reflecting the effect of residual ionization of the dimethylamino function (pK_a 7.7) in the reduction process.

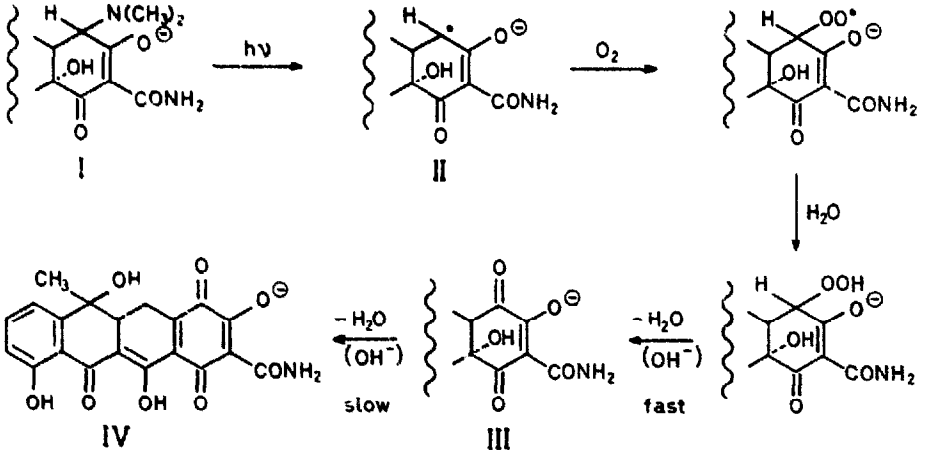
From Fig. 1 it can be seen that upon irradiation at pH 9.0 there was a decrease in height of both waves, but E_2 decreased at a faster rate than E_1, both eventually disappearing. The decrease in E_2 followed approximately the same rate as the oxygen consumption (moles oxygen per mole of tetracycline) and the decrease of absorbance at 373 nm (A_{373}).

The kinetic relationship between these 4 parameters is shown in Fig. 2 for the early stages of the reaction. The correlation between the decrease in A_{373} and E_2 holds throughout the reaction, but the oxygen uptake increases disproportionately as secondary reactions occur after about 50% reaction. The rate-determining factor is the intensity of the irradiating light. Similar changes in the polarogram and spectrum are evident but at a much slower rate when samples of tetracycline in buffer solution at pH 9.0 remain exposed to fluorescent room lighting.

The significant change in the polarogram caused by the irradiation is the appearance of a strong new wave at −0.130 V, indicative of a readily reducible species, such as a quinone. For a comparison, 1,4-benzoquinone and 1,4-naphthoquinone yield single reduction waves at −0.050 and −0.240 V, respectively, at pH 9.0.

Davies et al. (1979) found that the photo-oxidation reaction occurs in two stages, which can be distinguished by irradiation at low temperature. The postulated mechanism shown in Scheme 1 proposes that the photodissociation of the 4-dimethylamino group is rapidly followed by reaction with O_2, then H_2O elimination to form product III. The slower reaction leading to the quinone IV is a base-catalyzed dehydration, not dependent on light. In order to slow the latter thermal reaction we performed irradiations of air-saturated solutions at pH 9.0 in a vessel thermostatted down to 3°C. No quinone-like wave was seen except when the polarogram was taken

SCHEME 1



after the solution had warmed up to room temperature when the red colour was also seen.

However, a broad wave was observed having two unresolved components at −0.70 and −0.86 V. This is what might be expected of a molecule such as III with a reducibility roughly between that of I and IV.

The changing polarogram in Fig. 1 can be interpreted as follows. The faster decrease in E_2 is due to the loss of the dimethylamino group, while the slower decrease in E_1 is due to the replacement of the keto-enol system by the extended conjugation in III. In the absorption spectrum, it was found that the 373 nm absorption of I had changed on irradiation at 3°C to a more complex but less intense combination of a band at 357 nm and a very weak visible region of absorption corresponding to a brown colour of the solution: the 530 nm band appeared after the solution had warmed up to room temperature.

In a further attempt to gain information on the initial phase of the mechanism in Scheme 1, we irradiated tetracycline hydrochloride in the absence of oxygen at pH 9.0 and 30°C. Both polarographic waves simultaneously decreased in height but at one-third the rate found in air-saturated solutions. Also a new single wave appeared

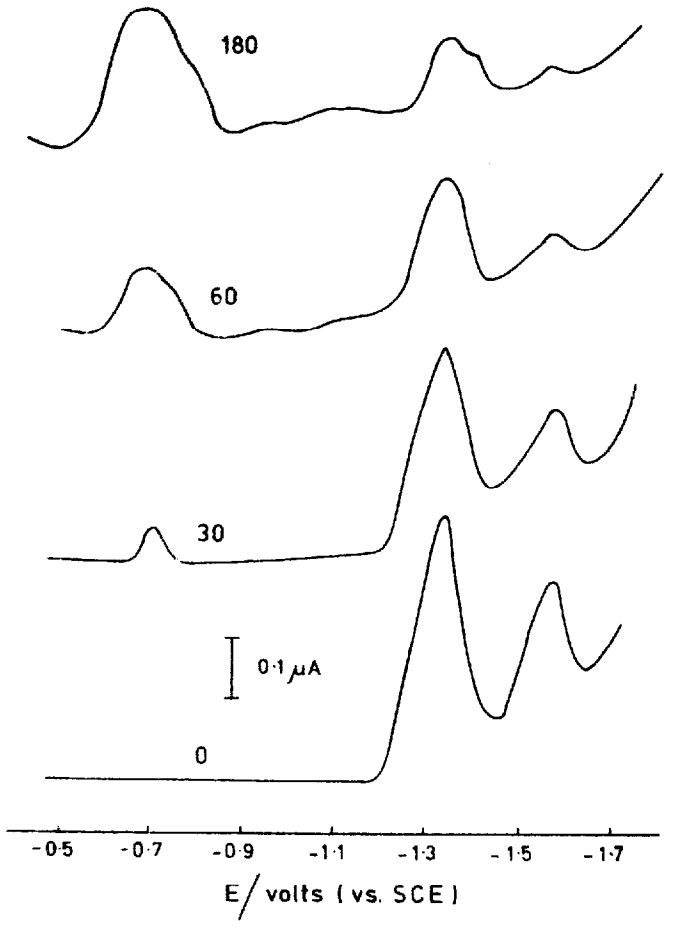
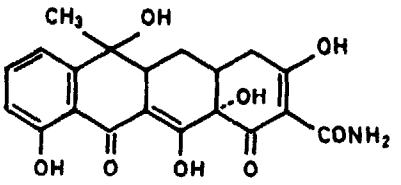


Fig. 3. Differential pulse polarograms recorded from tetracycline (5×10^{-5} M) in deoxygenated buffer solutions at pH 9.0 and 30°C irradiated for the times indicated (in min).

at −0.75 V as shown in Fig. 3. In the absorption spectrum, the 373 nm peak decreased as observed in air-saturated solution, but it also underwent a bathochromic shift, being at 405 nm after 2 h irradiation. This species was not stable to the effects of oxygen or further irradiation and is clearly different to that formed by the low temperature irradiations in air-saturated conditions.



V

In methanol solution, Hlavka and Bitha (1966) found that irradiation of tetracycline base produced 4-dedimethyl-aminotetracycline (V), independent of the presence of oxygen. Also they were able to collect and identify the volatile product dimethylamine. The mechanism they proposed involves formation of the radicals II and $(CH_3)_2\dot{N}$, both of which are presumed to abstract protons from solvent molecules. To follow this reaction polarographically we irradiated tetracycline base in methanol and took samples which were diluted in aqueous buffers at pH 5.0 and 9.0. The polarograms were recorded whereupon comparison with polarograms of an authentic sample of V confirmed V as the product of irradiation. There was no evidence of any other reduction wave. On the other hand, irradiation in deoxygenated aqueous solution yields a product whose polarogram does not correspond to that of V. The more easily reducible character of this product (reduction at −0.7 V) indicates the possible substitution of an OH in position 4.

The primary species postulated to occur in the photoreactions by both Hlavka and Bitha (1966) and Davies et al. (1979) are the free radicals II and $(CH_3)_2\dot{N}$. A method used successfully to detect the production of free radicals from irradiated compounds is the initiation of the polymerization of acrylamide (Williams, 1968). By this technique, one can identify compounds which are capable of sensitizing photo-oxidation by the Type I (free radical) mechanism. The alternative or Type II mechanism proceeds by excited state energy transfer involving singlet molecular oxygen, and can be recognized by the ability of known singlet oxygen quenchers to inhibit the reaction (Spikes, 1977).

Thus it was demonstrated that photosensitizers producing a high yield of relatively long-lived radicals, such as chlorpromazine, generate high rates of polymerization (Moore, 1977; Moore and Burt, 1981). Other compounds such as quinine and anthracene, which are efficient sensitizers of photo-oxidation, act predominantly by the Type II mechanism and give very low rates of polymerization due to a low yield of cation radicals by photoionization upon irradiation (Moore, 1980; Moore and Burt, 1981; Moore and Hemmens, 1982).

Davies et al. (1979) tested tetracycline photo-oxidation for singlet oxygen participation with negative results, and we have confirmed their observations using sodium azide as singlet oxygen quencher. They also tested for free radicals by adding

TABLE 1

PHOTOPOLYMERIZATION AND PHOTO-OXIDATION SENSITIZED BY TETRACYCLINE (TC) IN BUFFER AND SURFACTANT SYSTEMS AT 30°C

System	Polymerization rate [a] ($mmol \cdot l^{-1} \cdot min^{-1}$)		Photo-oxidation rate [b] ($\mu mol \cdot l^{-1} \cdot min^{-1}$)		Fluorescence intensity [c]
	pH 7.0	pH 9.0	TCalone	TC + DMF	
Buffer only	< 0.01	< 0.01	0.4	0.9	100
Cetrimide (0.01 M)	0.14	0.02	1.7	6.8	1230
Cetamacrogol (0.01 M)	0.07	0.01	0.5	1.5	140
SDS (0.05 M)	0.10	0.01	1.2	3.1	275
Methanol	< 0.01		0.1	3.4	N.D. [d]

[a] Initial rate of conversion of acrylamide to polymer: [acrylamide] = 0.125 M; [TC] = 8.2×10^{-5} M in all experiments.

[b] Initial rate of oxygen uptake from solution at pH 7: [DMF] = 8.6 mM: [TC] = 4.3×10^{-5} M in all experiments.

[c] Fluorescence intensity in arbitrary units: [TC] = 4.3×10^{-5} M in all solutions, measured at 25°C and pH 7.

[d] N.D. = not determined.

propan-2-ol, a good H atom donor, but found no influence on the formation of IV. We employed the polymerization technique by irradiating various tetracycline solutions with acrylamide in the dilatometer. The rate was measured in terms of contraction in the volume of solution by movement of the meniscus down the capillary tube. The solutions were de-aerated as oxygen is an inhibitor of polymerization, being a more efficient scavenger of free radicals than is acrylamide. The results, given in Table 1, show that no polymerization could be detected in aqueous buffer at pH 7.0 and 9.0, nor in methanol using both tetracycline free base and hydrochloride. However, in the surfactant solutions at concentrations where micelles were present—cetrimide (cationic), sodium dodecyl sulphate (SDS—anionic) and cetamacrogol (non-ionic)—small but significant reaction rates were measured. It is interesting to note that the polymerization rates were higher at pH 7.0 compared to pH 9.0 although the higher pH is responsible for the greater rate of oxidative degradation (Wiebe and Moore, 1977).

These results are to be considered on the basis that the measured polymerization rates are determined not only by the yield of initiating radicals but also their lifetimes (Williams, 1968).

Our interpretation of this data is that in simple aqueous solution any free radical formed from the tetracycline reacts rapidly with oxygen or water when under deoxygenated conditions. In surfactant solutions, the tetracycline molecules are incorporated into micelles to varying extents according to the type of surfactant and the state of ionization of the tetracycline. Such a varying level of incorporation into the micelles can be deduced from fluorescence measurements which show enhanced intensity in line with the increase in rate of reaction (Table 1). Thus the radicals formed on irradiation are stabilized to a certain extent within the micelles, long

enough to promote encounters with acrylamide molecules to initiate polymerization. Enhancement of free radical lifetimes by surfactants has been reported for chlorpromazine, frusemide and anthracene (Moore and Burt, 1981).

An increased lifetime of photo-excited tetracycline in the micelle is also indicated by faster rates of photo-oxidation of tetracycline by itself and with the addition of the oxidizable acceptor, 2,5-dimethylfuran (DMF), as shown in Table 1.

The differences in rates observed in different surfactants and pH 7.0 and 9.0 are principally a reflection of the electrostatic interactions between the tetracycline molecule and the charged surfactant (Fendler and Fendler, 1975). Tetracycline has a complex ionization pattern, with $pK_1 = 3.30$, $pK_2 = 7.68$ and $pK_3 = 9.69$ (Stephens et al., 1956). A zwitterionic structure was originally proposed for pH 7, with enolate anion at position 3 and dimethylammonium cation at position 4.

Recently, however, Smyth et al. (1978) suggested that an unionized structure of the molecule is more likely at this pH. Our results indicate that the charge of the surfactant is important, with the cationic surfactant the most effective and the non-ionic the least in interacting with tetracycline, thus suggesting the zwitterion. The fact that reaction rates with surfactants at pH 9 were not as high as at pH 7 is attributed to deprotonation of the dimethylammonium group with the formation of the monoanion of tetracycline which is less successfully incorporated into the micelles.

It is also possible that the proximity of the enolate anion at position 3 facilitates by intramolecular base catalysis the involvement of water molecules in the reactions occurring at position 4. This would also have the effect of protecting the free radical from reaction with acrylamide, thus accounting for the low rates of polymerization and the failure to detect free radicals in simple aqueous solution.

Our polarographic and polymerization measurements therefore provide further evidence to support the mechanism of Scheme 1 as proposed by Davies et al. (1979) for the photochemical oxidation of tetracycline in alkaline media. The differential pulse polarographic method is also recommended for the analysis of tetracycline samples. Small amounts of degradation products can be detected in the polarograms at potentials markedly different from the tetracycline reduction waves as seen in Figs. 1 and 3.

Several other tetracyclines were tested, including oxytetracycline, doxytetracycline, chlortetracycline, demethylchlortetracycline, methacycline and minocycline. In all cases the polarograms at pH 9.0 were dominated by the E_1 wave at about -1.3 V corresponding to the keto-enol system of ring A.

The appearance of other waves corresponding to E_2 of tetracycline appeared to be determined by the pK of the dimethylammonium function and hence the extent of its ionization. Irradiation produced changes similar to those described for tetracycline but with kinetics as expected from the oxygen uptake rates (Wiebe and Moore, 1977). Quinone-type waves were seen for all except chlortetracycline and demethyltetracycline which is in accord with the results of Davies et al. (1979).

References

Caplis, M.E., Ragheb, H.S. and Schall, E.D., Determination of tetracycline antibiotics by alternating-current polarography. J. Pharm. Sci., 54 (1965) 694–698.

Cutie, A.J., Mills, J. and Jochsberger, T., Analysis of mixtures of demeclocycline, minocycline and tetracycline utilizing differential pulse polarography. Drug Dev. Ind. Pharm., 6 (1980) 77–85.

Davies, A.K., McKellar, J.F., Phillips, G.O. and Reid, A.G., Photochemical oxidation of tetracycline in aqueous solution. J. Chem. Soc. Perkin Trans. II, (1979) 369–375.

Fendler, J.H. and Fendler, E.J., Catalysis in Micellar and Macromolecular Systems, Academic Press, New York, 1975, pp. 194–206.

Hlavka, J.J. and Bitha, P., Photochemistry IV. A photodeamination. Tetrahedron Lett., (1966) 3843-3846.

Jochsberger, T., Cutie, A.J. and Mills, J., Differential pulse polarography of tetracycline: determination of complexing tendencies of tetracycline analogs in the presence of cations. J. Pharm. Sci., 68 (1979) 1061–1063.

Magnus, I.A., Dermatological Photobiology, Blackwells, London, 1976, pp. 213–216.

McCormick, J.R.D., Fox, S.M., Smith, L.L., Bitler, B.A., Reichenthal, J., Origoni, V.E., Muller, W.H., Winterbottom, R. and Doerschuk, A.P., Studies of the reversible epimerization occurring in the tetracycline family. The preparation, properties, and proof of structure of some 4-epi-tetracyclines. J. Am. Chem. Soc., 79 (1957) 2849–2858.

Moore, D.E., Photosensitization by drugs. J. Pharm. Sci., 66 (1977) 1282–284.

Moore, D.E., Photosensitization by drugs: quinine as a photosensitizer. J. Pharm. Pharmacol., 32 (1980) 216–218.

Moore, D.E. and Burt, C.D., Photosensitization by drugs in surfactant solutions. Photochem. Photobiol., 34 (1981) 431–439.

Moore, D.E. and Hemmens, V.J., Photosensitization by antimalarial drugs. Photochem. Photobiol., 36 (1982) 71–78.

Oliff, C.J. and Chatten, L.G., AC polarography for tetracycline analysis. J. Pharm. Sci., 66 (1977) 1564–1566.

Smyth, M.R. and Smyth, W.F., Voltammetric methods for the determination of foreign organic compounds of biological significance. Analyst, 103 (1978) 529–567.

Smyth, W.F., Jenkins, T.C., Smyth, M.R., Barrett, J. and Sprake, S., Acid base equilibria in aqueous solutions of tetracycline hydrochloride. Acta Chem.Scand., B32 (1978) 131–136.

Spikes, J.D., Photosensitization. In Smith, K.C. (Ed.) The Science of Photobiology, Plenum Press, New York, 1977, p. 91.

Stephens, C.R., Murai, K., Brunings, K.J. and Woodward, R.B., Acidity constants of the tetracycline antibiotics. J. Am. Chem. Soc., 78 (1956) 4155–4158.

Wiebe, J.A. and Moore, D.E., Oxidation photosensitized by tetracyclines. J. Pharm. Sci., 66 (1977) 186–189.

Williams, F., Radiation induced polymerization. In Ausloos, P. (Ed.), Fundamental Processes in Radiation Chemistry, Interscience Publishers, New York, 1968, pp. 515–598.