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Pulse radiolysis of tetrazolium violet in aqueous and aqueous-alcoholic solutions under oxidative and reductive conditions

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ABSTRACT

The radiolytic reduction of colourless tetrazolium salts to coloured formazans in liquid and solid state is suggested for dosimetry purposes. In order to clarify the reaction mechanism, a pulse radiolysis study was conducted in aqueous and aqueous-alcoholic solutions under oxidative and reductive conditions. Under reducing conditions, fast formation of the electron adduct tetrazolinyl radical was observed: coloured formazan final product formed during the decay of electron adduct. Both the decay of the tetrazolinyl radical and the formation of the formazan were found to be second order. The spectra of the formazan were similar in neutral and alkaline solutions, but with higher absorbance in the latter solutions due to the higher molar absorption coefficient. Under oxidative conditions formazan did not form; hydroxylated products through OH-adducts were observed in the pH range studied.

1. Introduction

The heterocyclic organic tetrazolium salts are known to form highly coloured (pink, violet, red, lilac and blue) formazans upon reduction: this colour formation can be used for radiation dosimetry purposes. The reduction of the tetrazolium ring is a two-electron process (in ditetrazolium salts it is a four-electron process) (Altman, 1976).

Several members, mono- and ditetrazolium salts of the tetrazolium family, have been investigated in solutions or in solid state for characterization as dosimeters (Kovács et al., 1996, 1999b; Kriminskaya et al., 1987b, 1992; Pikaev and Kriminskaya, 1998). In a few cases mechanistic studies were also performed using ESR spectroscopy at low temperatures (Kriminskaya et al., 1987a, 1991). Pulse radiolysis studies have been conducted in aqueous and aqueous-alcoholic (ethanol, isopropanol) solutions of tetrazolium molecules containing three phenyl substituents (2,3,5-triphenyl-tetrazolium chloride, TTC) (Kriminskaya et al., 1984, 1988a, b; Kovács et al., 1996), two phenyl and one *p*-nitrophenyl substituent (2-*p*-nitrophenyl-3.5-diphenyltetrazolium chloride (p-NTC) (Kriminskaya et al., 1988a) and with a ditetrazolium compound having phenyl, *p*-nitrophenyl and *m*-methoxyphenyl substituents (nitro blue tetrazolium chloride, NBTC) (Bielski et al., 1980; Kovács et al., 1999a). It was shown that under reducing conditions in aqueous solutions the solvated electron, while in aqueous-alcoholic solutions both the solvated electron and the α-hydroxyalkyl radicals reduce the tetrazolium ring into the electron adduct tetrazolinyl radical. These radicals either disproportionate, resulting in formazans, or dimerize.

In a recent paper we showed the potential applicability of tetrazolium violet (TV) for dosimetry purposes in aqueous and aqueous-alcoholic solutions (Emi-Reynolds et al., 2007a) and also in polyvinylalcohol-based radiochromic dye films (Emi-Reynolds et al., 2007b). This compound has two phenyl and one α-naphthyl substituent attached to the tetrazolium ring. Since the α-naphthyl substituent through the electron conjugation may influence strongly the spectral and kinetic characteristics of the intermediates, and also the reaction mechanism, using pulse radiolysis we carried out mechanistic investigations.

2. Experimental

The tetrazolium violet salt (2,5-diphenyl-3-(1-naphthyl)-2H-tetrazolium chloride ($C_{23}H_{17}N_4Cl$) from Aldrich was used without further purification. The aqueous solutions were prepared with double-distilled water and bubbled with N_2 or N_2O, respectively. The *tert*-butanol and ethanol were from Reanal and were used as obtained. The pH of the solutions was set with $HClO_4$ and NaOH, respectively, both from Reanal.

The pulse radiolysis experiments were carried out with the LPR-4 linear accelerator (Tesla Vuvet, Czech Republic) and the

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connected optical detection system for kinetic spectrophotometry of the Institute of Isotopes with 2.6 µs electron pulses (Földiák et al., 1988). The dose per pulse was measured with KSCN solution and it was generally ~30 Gy pulse^{-1}.

3. Results and discussion

3.1. Radiolysis of TV under reducing conditions

In order to study the behaviour of aqueous tetrazolium violet solution under reducing conditions, the oxidizing OH radicals of water radiolysis have been eliminated from the solution by the addition of *tert*-butanol. N_2 purging was used in order to get rid of the oxygen. In such solutions the *hydrated electron and H atom* with *G*-values of 2.7 and 0.6 species per 100 eV energy absorbed are the reactive species.

The absorption spectrum, taken 14 µs after the electron pulse in 0.1 mmol dm^{-3} TV containing acidic (pH = 3.3; 5.7), neutral (pH = 6.7) and alkaline (pH = 10.8) aqueous solutions, is similar and shows a strong absorption band in the 300–600 nm region with a maximum at 350 nm and a smaller absorption peak at around 430 nm, followed by a decreasing absorption up to about 600 nm (Fig. 1). On a longer time scale, i.e. 0.6 and 1.8 ms after the electron pulse, however, both absorption bands at 350 and 430 nm get significantly smaller. The decrease of the absorption in the 450–580 nm region in neutral and acidic solutions on the ms time scale is much smaller, than at the lower wavelengths. In alkaline solutions an increase in the absorbance appears in this wavelength region. It means that a new absorbing species forms with maximum absorbance at around 450–500 nm (Fig. 2).

Taking into account the results obtained in the case of other tetrazolium salts also (e.g. 2,3,5-triphenyl-tetrazolium chloride, 2-nitrophenyl-3.5-diphenyl-tetrazolium chloride, nitro blue tetrazolium chloride) the fast formation of such species—i.e. the build-up of absorbance at 350 nm and at around 430 nm within a few µs—is attributed to the formation of the electron adduct tetrazolinyl radical, where the tetrazolium ring has undergone one-electron reduction:

$$\mathrm{TV} + {+e_{aq}^{-}} \xrightarrow{k} \mathrm{TV}^{\bullet} \qquad (1)$$

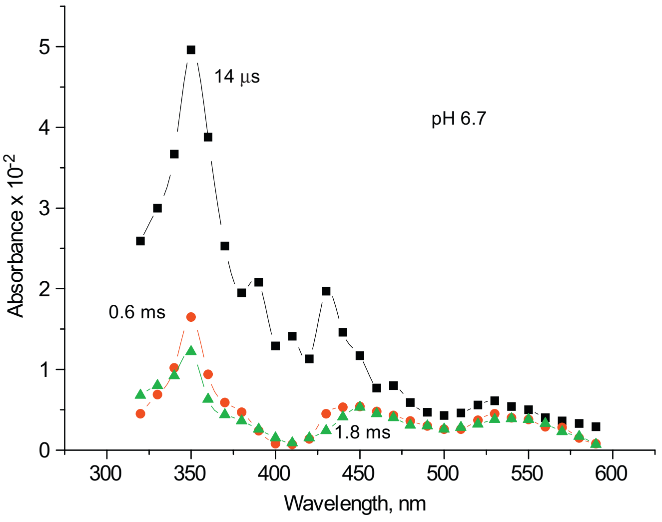

Fig. 1. Optical absorption spectra in N_2-saturated aqueous solutions of 0.1 mmol dm^{-3} of TV containing *tert*-butanol taken 14 µs (■), 0.6 ms (●) and 1.8 ms (▲) after the electron pulse (pH 6.7).

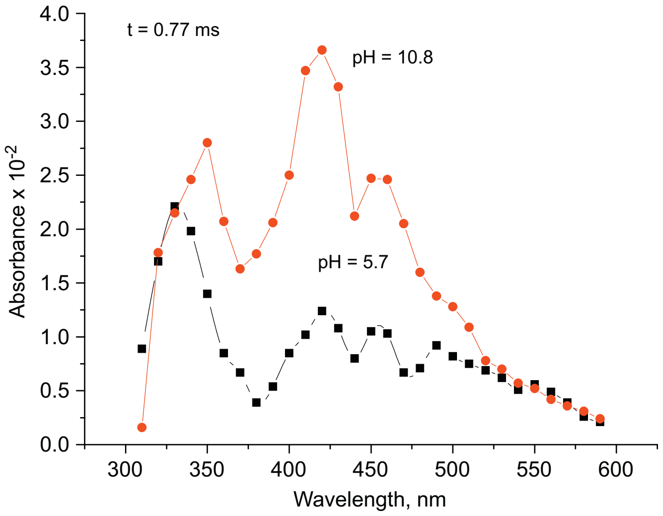

Fig. 2. Optical absorption spectra taken at 0.77 ms after the electron pulse in slightly acidic and alkaline N_2-saturated, *tert*-butanol containing aqueous solutions of 0.1 mmol dm^{-3} TV.

Table 1
Spectral characteristics of intermediate species (tetrazolinyl radicals) and final products (formazans) of some tetrazolium salts

Tetrazolium salt	Tetrazolinyl radical, λ (nm)	Formazan, λ (nm)
TTC	360	480
NBTC	420	530
TV	350	420
p-NTC	415	480

The spectral characteristics of the intermediate (tetrazolinyl radical) and final (formazan) radiolysis products of some tetrazolium salts are shown in Table 1.

Since the TV concentration is higher by about three orders of magnitude than the hydrated electron concentration produced during the pulse, the reduction from the kinetics point of view is a pseudo-first-order process. The second-order rate coefficient was calculated to be about 10^{10} dm^3 mol^{-1} s^{-1}. This value is close to the values published in the literature for similar molecules (Kriminskaya et al., 1988a; Lecheheb et al., 1984).

As already mentioned above in N_2 saturated *tert*-butanol containing, neutral and especially in alkaline solutions a broad absorption band appears in the 450–550 nm region on the hundreds of µs and ms time scale. It is reasonable to assume that the tetrazolinyl radicals disappear in the second-order process (Fig. 2) in self-termination reaction, resulting in the formation of monoformazan. Self-termination of the tetrazolinyl radicals was also suggested in the case of other tetrazolium compounds (Kriminskaya et al., 1988a; Kovács et al., 1999a):

$$\mathrm{TV}^{\bullet} + \mathrm{TV}^{\bullet} + \mathrm{H}^{+} \rightarrow \mathrm{TV}^{+} + \mathrm{MF} \qquad (2)$$

The absorbance build-up of the formazan final product is also shown in Fig. 3, both the tetrazolinyl radical decay and the formazan build-up yielded the same rate coefficient of 1×10^9 dm^3 mol^{-1} s^{-1}. The same value was found for the decay of the tetrazolinyl radical produced in 2,3,5-triphenyl-tetrazolium chloride solution in our former publication (Kovács et al., 1996).

The absorption spectrum of formazan is similar at all pH values studied. It is important to note, however, that on a longer time scale (0.77 ms) much stronger absorption was observed in alkaline solutions at 420 nm and in the 450–550 nm region, respectively,