Russian Chemical Reviews **67** (8) 671 – 680 (1998) © 1998 Russian Academy of Sciences and Turpion Ltd

UDC 541.15

Radiolysis of solutions of tetrazolium salts

A K Pikaev, Z K Kriminskaya

Contents

I.	Introduction	671
II.	Alcoholic solutions	671
III.	Aqueous and water – alcohol solutions	674
IV.	Solid matrices	676
V.	The use of tetrazolium salts in dosimetry	677
VI.	Conclusion	679

Abstract. Published data on radiolysis of liquid (mainly aqueous, water – alcohol and alcoholic) and solid [mainly in poly(vinyl alcohol)] solutions of tetrazolium salts are generalised. The mechanism of radiolytic transformations of these compounds is discussed. Their application in dosimetry of ionising radiation is considered in detail. The bibliography includes 60 references.

I. Introduction

Reduction of colourless or weakly coloured tetrazolium salts yields formazans, which are intensely coloured. This property of formazans has found application in various fields of science and engineering. They have been used in botany as colouring compounds sensitive to ultraviolet radiation,[1] as substances suitable for estimating the productivity of seeds of agricultural plants [2,3] and the viability of stem and leaf tissues,[4,5] for distinguishing between normal and malignant tissues,[6] and for studying the histochemistry of oxidative enzymes.[7,8] The use of formazans as analytical reagents for detection of O_2^- radical ions has also been reported.[9–11] In addition, it is known (see, for example, Ref. 12) that liquid and solid solutions of formazans can be employed in the dosimetry of ionising radiation.

The properties, methods for the synthesis, and the mechanism of chemical reduction of tetrazolium salts have been considered in numerous publications (see, for example, Refs 13 – 17). The present review is mainly devoted to radiolytic transformations of these salts in various solvents (alcohols, water, aqueous alcohols, *etc.*). Solutions of 2,3,5-triphenyltetrazolium chloride (TPTC) have been studied most often; solutions of 2-(*p*-nitrophenyl)-3,5-diphenyltetrazolium chloride (NPTC), 5-(*p*-nitrophenyl)-2,3-diphenyltetrazolium bromide (NPTB), 3-(1-naphthyl)-2,5-diphenyltetrazolium bromide (DPTB), 3,3′-bianisylene-4,4′-bis(2,5-diphenyl-2*H*-tetrazolium) chloride (Tetrazolium Blue, TB), 2,2′-di(*p*-nitrophenyl)-5,5′-diphenyl-3,3′-(dimethoxy-4,4′-diphenylene)-2*H*-tetrazolium chloride (Nitrotetrazolium Blue, NTB) and some other derivatives have been studied more rarely. Considerable attention is paid to the use of these systems in the dosimetry of ionising radiation.

A K Pikaev Institute of Physical Chemistry, Russian Academy of Sciences, Leninskii prosp. 31, 117915 Moscow, Russian Federation.
Fax (7-095) 335 17 78. Tel. (7-095) 333 95 67
Z K Kriminskaya Russian Research Centre 'Research Institute of Organic Intermediates and Dyes', Bol'shaya Sadovaya ul. 1, 103787 Moscow, Russian Federation. Fax (7-095) 254 12 00

Received 28 October 1997
Uspekhi Khimii **67** (8) 745 – 754 (1998); translated by Z P Bobkova

II. Alcoholic solutions

Detailed data on the radiation yields of reduction of tetrazolium salts in alcoholic solutions and on the mechanism and kinetics of this process can be found in the literature.

1. Radiation yields of reduction

When colourless deaerated alcoholic solutions of tetrazolium (T^+) salts are exposed to radiation, the corresponding intensely coloured (pink, orange-red, or red) formazans are produced. For example, in the case of TPTC, this yields 1,3,5-triphenylformazan PhNH – N = CPh–N = NPh.

The radiation yields of formazans (F) in these solutions are listed in Table 1.

2. The mechanism of radiolytic reduction

Radiolytic reduction of tetrazolium salts is a two-electron process, *i.e.* the reduction of each tetrazolium salt molecule requires two reducing species. The yield of the formazan in this process is more than half the yield of solvated electrons (e_s^-) (for example, in isopropanol, the yield of e_s^- is 1.3 [26]). Based on this result, it was concluded that the salts are reduced by not only e_s^- but also by radicals of type $\dot{R}CHOH$ resulting from radiolysis of alcohols. When the doses are large enough, formazans undergo subsequent radiolytic transformations; the solutions become colourless, and hydrazidines are formed. The yields of decomposition of formazans have been determined for the radiolysis of solutions of 1,3,5-triphenylformazan,[18,27] 3-(*p*-nitrophenyl)-1,5-diphenylformazan,[20] and 2,5-diphenyl-3-(1-naphthyl)formazan [20] in isopropanol (Table 2).

The conclusion about two-electron reduction of tetrazolium salts is confirmed by the data [19,28,29] on pulse radiolysis of solutions of these compounds in ethanol and isopropanol. It was found that the interaction of the tetrazolium salt cation (T^+) with e_s^- or with an alcohol radical gives a short-lived species, namely tetrazolium radical ($T^{\bullet}$). In the case of TPTC, this radical has the following structure:

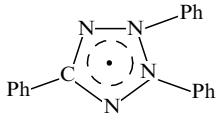


Table 1. Yields of formazans [G(F), molecule (100 eV)$^{-1}$] in various matrices upon γ-radiolysis in the absence of air.

Matrix	Tetrazolium salt	10^3 c/ mol litre^{-1}	G(F)	Ref.
Liquid isopropyl alcohol	TPTC	0.1	1.3	18
	TPTC	0.5	1.5	18
	TPTC	1.0	1.5	18
	TPTC	5.0	1.7	18
	TPTC	7.0	2.2	18
	NPTC	0.1	1.5	19
	NPTC	0.5	2.5	19
	NPTC	1.0	2.5	19
	NPTC	5.0	2.5	19
	NPTB	0.1	2.2	19
	NPTB	0.5	3.3	19
	DPTB	0.1 – 5	3.1	20
Water, pH				
6.9	TPTC	1.0	0.45	21
7.5 [a]	TPTC	20	0.4	22
10.3	TPTC	1.0	1.2	21, 23
6.9	NPTC	1.0	0.6	21
10.3	NPTC	1.0	1.4	21
6.9	NPTB	0.5	1.2	21
10.3	NPTB	0.5	1.2	21
5.3	DPTB	1.0	0.6	20
10.0	DPTB	1.0	0.7	20
11.5	DPTB	1.0	0.8	20
Water – isopropanol (2.2 mol litre^{-1}), pH 10.3	TPTC	1.0	3.5	21
Water – isopropanol (20%), pH 11	TPTC	0.8	3.1	23
Water – isopropanol (1 : 1 by volume), pH 11	TPTC	0.8	3.1	24
Water – HCOONa (0.2 mol litre^{-1}), pH 10.3	TPTC	1.0	3.0	21
Water – EDTA (0.2 mol litre^{-1}), pH 10.3	TPTC	1.0	3.1	21
Poly(vinyl alcohol) film	TPTC	7.4	0.86	25
	TPTC	11	2.6	25
	TPTC	18	3.1	25
	NPTC	3.2	2.8	25
	NPTC	9.8	4.0	25
	NPTC	13	4.8	25
	NPTC	16	5.2	25

[a] For radiolysis induced by X-rays (the highest energy 60 keV). The solution was buffered by triethanolamine and HCl.

Table 2. Yields of decomposition of formazans upon γ-radiolysis of deaerated isopropanol solutions.

Formazan	10^4 c / mol litre^{-1}	G/ molecule (100 eV)$^{-1}$	Ref.
1,3,5-Triphenyl-formazan	1.4	3.5	18, 27
3-(*p*-Nitrophenyl)-1,5-diphenylformazan	5.8	3.1	20
2,5-Diphenyl-3-(1-naphthyl)formazan	0.27	1.3	20
	0.77	2.4	20

A similar species is formed as an intermediate during the chemical reduction of these compounds (see, for example, Refs 3 and 17).

The structure of the tetrazolium radical has been confirmed by the results of EPR studies of irradiated frozen solutions of TPTC and 2,3,5-triphenyltetrazolium tetrafluoroborate.[30,31] It was found that irradiation of these solutions at 77 K affords solvated electrons and alcohol radicals. During the subsequent fast warming up to room temperature, these species react with the tetrazolium salt yielding tetrazolium radicals. The EPR spectra of the radicals recorded after cooling the samples to 77 K are identical for these two salts and consist of eight lines with a splitting of ~1.4 mT.

A similar EPR spectrum has been recorded at 77 K for the $T^{\bullet}$ radical resulting from reduction of TPTC with sodium ethoxide in isopropanol. The spectrum recorded at 300 K is somewhat different. Due to the anisotropy of the hyperfine coupling tensor, the spectrum of $T^{\bullet}$ in the liquid phase is characterised by smaller splitting (~0.7 mT).[31]

The data obtained in studies on pulse radiolysis of solutions of tetrazolium salts [19,28,29] indicate that tetrazolium radicals arise in two processes, one of which is fast and the other is slow. For example, in the case of a triphenyltetrazolium salt (the TPT^+ cation) in isopropanol, this might occur as follows:

$$TPT^+ + e_s^- \xrightarrow{k_1} TPT^{\bullet}, \quad (1)$$

$$TPT^+ + (CH_3)_2\dot{C}OH \xrightarrow{k_2} TPT^{\bullet} + (CH_3)_2CO + H^+. \quad (2)$$

Table 3 contains the rate constants for reactions of this type for tetrazolium salts measured by the method of pulse radiolysis. Calculations using the Debye equation have shown that in the case of NPTC and NPTB, the rate of reaction (1) is controlled by diffusion, whereas for TPTC, the rate is lower than the diffusion limit.

The formazans are formed upon disproportionation of $T^{\bullet}$ radicals

$$T^{\bullet} + T^{\bullet} + ROH \xrightarrow{k_3} F + T^+ + R^-. \quad (3)$$

Thus, the yield of the formazan (for example, in an isopropanol solution) should be equal to

$$G(F) = \frac{1}{2}\{G(e_s^-) + G[(CH_3)_2\dot{C}OH]\}. \quad (4)$$

Since $G[(CH_3)_2\dot{C}OH]$ in isopropanol is ~5.5 molecule (100 eV)$^{-1}$,[26] the theoretical G(F) value amounts to ~3.4 molecule (100 eV)$^{-1}$. Experimental yields are close to this value only for NPTB and DPTB (see Table 1). For TPTC and NPTC, the yields are substantially smaller. This is due to the fact that the tetrazolium radicals derived from these salts participate not only in reaction (3) but also in another reaction, which does not lead to formazan (for example, they can dimerise to give ditetrazolium salt TT).[18,19]

$$T^{\bullet} + T^{\bullet} \xrightarrow{k_5} TT. \quad (5)$$

According to our estimates,[18] the k_3/k_4 ratio for a solution of TPTC in isopropanol is ~1.9.

Tetrazolium salts dimerise relatively easily at the 4,4′-diphenyl bridge.[36] Nitrotetrazolium Blue is an example of this type of dimer. Ditetrazolium compounds have also been described in a review.[17]

Solutions of tetrazolium radicals in alcohols are characterised by intense optical absorption in the visible and ultraviolet spectral regions (Table 4). These radicals are relatively long-lived species, their lifetimes being of the order of milliseconds.

The reduction of formazan to hydrazidine (HH) is also a two-electron process.[26] It has been found by pulse radiolysis [27] that formazan reacts first with a solvated electron and an alcohol radical. The rate constants for these reactions in isopropanol are presented in Table 3. It was postulated [27] that the above reactions

Table 3.Rate constants [k/ litre mol^{-1} s^{-1}] for reactions of tetrazolium salts and formazans measured by pulse radiolysis.

Matrix	Tetrazolium salts	Reaction	k	Ref.	Matrix	Tetrazolium salt	Reaction	k	Ref.
Ethanol	TPTC	$TPT^+ + e_s^-$	4.1×10^9	29	water, pH				
	TPTC	$TPT^+ + CH_3\dot{C}HOH$	2.5×10^8	29	7	TPTC	$TPT^\bullet + O_2$	3.7×10^7	23
Isopropyl	TPTC	$TPT^+ + e_s^-$	2.1×10^9	28	7	NPTB	$NPT^\bullet + O_2$	4.5×10^7	23
alcohol	TPTC	$TPT^+ + (CH_3)_2\dot{C}OH$	3.1×10^8	28	6.9	TPTC	$TPT^\bullet + TPT^\bullet$	2.0×10^9	32
	NPTC	$NPT^+ + e_s^-$	1.5×10^{10}	19				(see [c])	
	NPTC	$NPT^+ + (CH_3)_2\dot{C}OH$	1.9×10^9	19	water	TPTC	$TPT^\bullet + TPT^\bullet$	1.0×10^9	35
	NPTB	$NPT^+ + e_s^-$	1.4×10^{10}	19				(see [c])	
	NPTB	$NPT^+ + (CH_3)_2\dot{C}OH$	1.7×10^9	19	water, pH 6.9	NPTC	$NPT^\bullet + NPT^\bullet$	1.8×10^9	32
	F [a]	$F + e_s^-$	3.3×10^{10}	27				(see [c])	
	F	$F + (CH_3)_2\dot{C}OH$	1.7×10^8	27	2	TPTC	$TPTH^{+\bullet} + TPTH^{+\bullet}$	5.3×10^7	32
Water, pH							(see [d])	(see [c])	
6.9	TPTC	$TPT^+ + e_{aq}^-$	1.1×10^{10}	32	2	NPTC	$NPTH^{+\bullet} + NPTH^{+\bullet}$	2.8×10^7	32
11.3	TPTC	$TPT^+ + e_{aq}^-$	6.7×10^9	32			(see [d])	(see [c])	
6.9	NPTC	$NPT^+ + e_{aq}^-$	1.1×10^{10}	32	5.7	NTB	$NTB^{+\bullet} + NTB^{+\bullet}$	3.8×10^8	33
11.3	NPTC	$NPT^+ + e_{aq}^-$	9.2×10^9	32	10	NTB	$NTB^{+\bullet}...OH^- +$	3.7×10^9	33
2	TPTC	$TPT^+ + H^\bullet$	$\sim1.4\times10^{10}$	32			$+ NTB^{+\bullet}...OH^-$		
2	NPTC	$NPT^+ + H^\bullet$	5.7×10^9	32	water	NTB	$NTB^{+\bullet} +$	4.9×10^9	33
6.9	TPTC	$TPT^+ + {}^\bullet OH$	7.1×10^9	32			$+ NTB^{+\bullet}...OH^-$		
6.9	NPTC	$NPT^+ + {}^\bullet OH$	1.0×10^{10}	32	6.9	TPTC	$TPTOH^{+\bullet} \rightarrow$	1.2×10^4	32
7.07 – 11.1	NTB	$NTB^{2+} + O_2^-$	5.9×10^4	33			$\rightarrow$ products	(see [f])	
			(see [b])	33			(see [e])		
10	TPTC	$TPT^+ + COO^{-\bullet}$	1.1×10^9	21, 34	6.9	NPTC	$NPTOH^{+\bullet} \rightarrow$	2.3×10^3	32
10	NPTC	$NPT^+ + COO^{-\bullet}$	5.0×10^9	21			$\rightarrow$ products	(see [f])	
10	NPTC	$NPT^+ + COO^{-\bullet}$	1.9×10^9	21			(see [e])		
10	NTB	$NTB^{2+} + COO^{-\bullet}$	6.4×10^9	33					

[a] F — 1,3,5-triphenylformazan. [b] The constant was determined by the stopped flow method. [c] The $2k$ value is given. [d] $TPTH^{+\bullet}$ and $NPTH^{+\bullet}$ are the H-adducts of the corresponding tetrazolium cations. [e] $TPTOH^{+\bullet}$ and $NPTOH^{+\bullet}$ are the OH-adducts of the corresponding tetrazolium cations. [f] Expressed in s^{-1}.

Table 4. Optical characteristics of the intermediate products of radiolysis of solutions of tetrazolium salts.

Matrix	Tetrazolium salt	Product	λ_{max}/ nm	$10^{-2}\,\varepsilon$/ m^2 mol^{-1}	Ref.
Ethanol	TPTC	$TPT^\bullet$	< 375, 400 (shoulder)	–	29
Isopropyl alcohol	TPTC	$TPT^\bullet$	365, 400 (shoulder)	10.7, 4.0	19, 28
	NPTC	$NPT^\bullet$	405, 620	9.9, 2.5	19
	NPTB	$NPT^\bullet$	370, 390 (shoulder)	6.3, 2.5	19
Water, pH 6.9, 11	TPTC	$TPT^\bullet$	370	7.1	32
Water	TPTC	$TPT^\bullet$	360	–	35
Water, pH 6.9, 11	NPTC	$NPT^\bullet$	415	7.6	32
5.7 – 11	NTB	$NTB^{+\bullet}$	405	15	33
2, 6.9	TPTC	$TPTH^{+\bullet}$	380	4.6	32
2, 6.9	NPTC	$NPTH^{+\bullet}$	390	–	32
6.9	TPTC	$TPTOH^{+\bullet}$	365	4.0	32
Water	TPTC	$TPTOH^{+\bullet}$	345	–	35
Water, pH 6.9	NPTC	$NPTOH^{+\bullet}$	380	–	32

yield formazan radical anions $F^{-\bullet}$, which are responsible for an intense optical absorption band with a maximum at 700 nm (the molar extinction coefficient is 3.8×10^3 m^2 mol^{-1}). The lifetime of these species is relatively long (more than 1 – 2 ms). By analogy with the behaviour of other radical cations in alcoholic solutions (see, for example, Ref. 37), it was suggested that $F^{-\bullet}$ are protonated in the following way (the reaction is written for isopropanol)

$$F^{-\bullet} + (CH_3)_2CHOH \xrightarrow{k_6} FH^\bullet + (CH_3)_2CHO^- \quad (6)$$

and disproportionation of the resulting $FH^\bullet$ gives HH

$$FH^\bullet + FH^\bullet \xrightarrow{k_7} F + HH. \quad (7)$$

It should be noted that similar reactions occur during two-electron reduction of quinones to hydroquinones.[38]

Obviously, the yield of formazan reduction in isopropanol should be determined by the equation

$$G(-F) = \frac{1}{2}\{G(\bar{e_s}) + G[(CH_3)_2\dot{C}OH]\}. \quad (8)$$

The calculated value of $G(-F)$ is $\sim$3.4 molecule (100 eV)$^{-1}$, which coincides with experimental results for 1,3,5-triphenylformazan [27] and 3-(*p*-nitrophenyl)-1,5-diphenylformazan [20] (see Table 2). However, in the case of 3-(1-naphthyl)-2,5-diphenylformazan,[20] the experimental yield is much lower than the theoretical yield. This is apparently due to the fact that the rate constant for

the reaction of this formazan with the alcohol radical is relatively low.

It has been found [18] that the yield of formazan in the radiolysis of alcoholic solutions of tetrazolium salts decreases when the radiation doses are high. This effect is least pronounced for solutions of DPTB in isopropanol.[20] Evidently, the yield decreases due to reactions of F with e_s^- and $(CH_3)_2C^{\bullet}OH$. 2-Isobutylanthraquinone added to an isopropanol solution of TPTC largely 'protects' the formazan from decomposition.[18] The dependences of the concentration of the formazan formed in isopropanol solutions of TPTC on the radiation dose in the presence and in the absence of 2-isobutylanthraquinone are shown in Fig. 1.

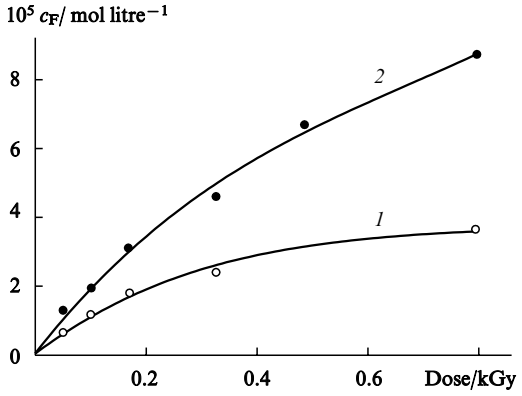


Figure 1. Concentration of the formazan formed *versus* the dose in a deaerated 3×10^{-3} M solution of TPTC in the absence (*1*) and in the presence (*2*) of 5×10^{-3} mol litre^{-1} of 2-isobutylanthraquinone.

3. Radiolysis of aerated solutions

The yield of formazans in aerated alcoholic solutions is appreciably lower than in deaerated solutions; in some cases, formazans are not formed at all. For example, they are not formed upon irradiation of air-saturated 1×10^{-3} M solutions of DPTB with a dose of 200–300 Gy.[20] According to another study,[39] the G(F) values for aerated 5×10^{-3} and 4.5×10^{-2} M ethanolic solutions of TPTC are 0.048 and 0.135 molecule (100 eV)$^{-1}$, respectively (at a dose of $\sim$10 kGy). It was found [22] that in the case of X-ray radiolysis of an aerated solution of TPTC in ethylene glycol (maximum energy 60 keV), G(F) increases with an increase in the concentration and reaches 1.03 molecule (100 eV)$^{-1}$ in a 0.1 M solution at a dose of 9.2 kGy.

This type of behaviour can be explained as follows. Since the concentration of TPTC is high, a large portion of tetrazolium radicals, generated, for example, according to reaction (1), are converted again into TPT^+.

$$TPT^{\bullet} + O_2 \xrightarrow{k_9} TPT^+ + O_2^- . \qquad (9)$$

A similar effect is also observed in the case of aqueous solutions of tetrazolium salts (see below). Yet another possible reason (although it is less significant) is that the alcohol radical might react with oxygen to give the peroxy radical $RCH(OH)O_2^{\bullet}$, and the latter reacts with TPTC slowly or not at all.

In an ethylene glycol solution, G(F) is close to 0.5 $G(e_s^-)$. Apparently, due to the high viscosity of this alcohol, reaction (9) in this system is relatively slow, and the $TPT^{\bullet}$ radicals generated mostly disproportionate. The alcoholic radicals do not reduce TPTC, but instead, they react with oxygen to give the peroxy radicals.

III. Aqueous and water–alcohol solutions

Radiolysis of aqueous and water–alcohol solutions of tetrazolium salts has been studied in several publications.[20–24,32,35] It was found that as in the case of alcoholic solutions, reduction to formazans is the prevailing process.

1. Characteristic features of radiolytic reduction in deaerated aqueous solutions

Irradiation of a deaerated aqueous solution of any of the tetrazolium salts studied results in the formation of a precipitate. The solution obtained by filtering this precipitate off and dissolving it in an alcohol, or by adding a sufficient amount of an appropriate organic solvent to the irradiated solution, or by extracting the product with a solvent exhibits an optical absorption spectrum typical of formazan. Upon irradiation of solutions in aqueous alcohols, when the alcohol concentration is high enough (for example, in a 1 : 1 water–isopropanol mixture [24]), the precipitate does not appear, but solution acquires an intense red colour caused by the formation of formazan. It should be noted that in aqueous solutions, the concentration of formazan arising upon radiolysis depends on the γ-radiation dose only at high doses, because the product precipitates. In aqueous alcohols, this dependence is sharper.

The measured G(F) values for radiolysis of deaerated solutions of some tetrazolium salts are listed in Table 1. It can be seen that without additives G(F) increases from 0.4 to 1.4 molecule (100 eV)$^{-1}$ with an increase in the pH of the solution from 5.3 to 11.5. However, in the case of NPTB, this dependence is not observed. Upon the addition of some organic compounds (formates, EDTA, isopropanol) or in water–isopropanol mixtures, the formazan yield increases to 3.5 molecule (100 eV)$^{-1}$.

The radiolysis of water is described by the following greneral scheme:[26]

$$H_2O \rightsquigarrow e_{aq}^-, H, OH, H_2O_2, H_2, H^+, OH^- . \qquad (10)$$

Obviously, the first four products can participate in the radiolytic transformations of tetrazolium cations. Hydrated electrons e_{aq}^- reduce the T^+ cations to tetrazolium radicals $T^{\bullet}$; the latter disproportionate to give formazan. Hydrogen atoms do not participate in the reduction,[32] but they add to benzene rings giving rise to H-adducts. The OH radical reacts with T^+ to give an OH-adduct. Hydrogen peroxide can oxidise $T^{\bullet}$.

$$T^{\bullet} + H_2O_2 \xrightarrow{k_{11}} T^+ + OH + OH^- . \qquad (11)$$

Subsequent transformations of the H- and OH-adducts give no formazans. Therefore, we can write

$$G(F) = \frac{1}{2}[G(e_{aq}^-) - G(H_2O_2)] . \qquad (12)$$

Calculation using this equation affords G(F) = 1.1 molecule (100 eV)$^{-1}$. The experimental G(F) values obtained for alkaline media (except for those for DPTB) are close to this predicted value. In the case of NPTB, the yields measured both in alkaline and neutral media coincide with those calculated from Eqn (12).

The experimental G(F) values found for acidic, neutral, and weakly alkaline solutions of TPTC and NPTC are smaller than the theoretical values. As an example, Fig. 2 shows the dependence of G(F) on the pH for a deaerated 1×10^{-3} M solution of TPTC.[34] At pH < 4, the formazan yield is close to zero, which is due to the fast reaction

$$e_{aq}^- + H^+ \longrightarrow H. \qquad (13)$$

The H atoms react with T^+ giving H-adducts.

Apparently, the $T^{\bullet}$ radicals derived from TPTC and NPTC not only disproportionate giving rise to formazan but also participate in some other processes, which do not lead to this

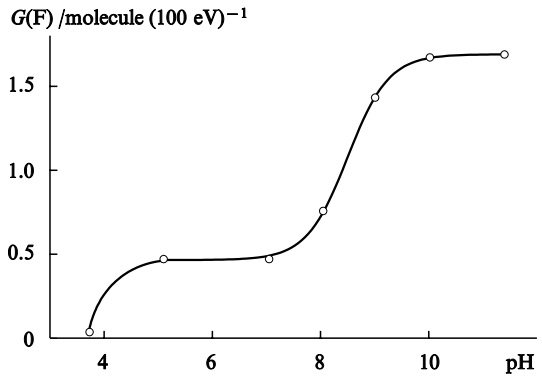


Figure 2. Dependence of $G(F)$ in a deaerated 1×10^{-3} M aqueous solution of TPTC on the pH.

compound (for example, they can dimerise).[32, 34] The tetrazolium radicals generated from NPTB do not tend to dimerise (apparently, due to steric hindrance). In alkaline media dimerisation does not occur either, probably due to the fact that the product formed upon the reaction of $T^{\bullet}$ with OH^- only disproportionates.[32] It was found by pulse radiolysis [32] that in TPTC solutions at pH > 9, when $G(F)$ increases (see Fig. 2), a new short-lived species appears in place of the $T^{\bullet}$ radicals consumed.

2. Radiolysis in the presence of organic compounds

Radiolysis of deaerated alkaline aqueous solutions containing organic compounds (formate, EDTA, alcohols) or solutions in water–alcohol mixtures results in $G(F)$ values reaching 3.5 molecule $(100\ eV)^{-1}$ (see Table 1). Evidently,[21] when the concentration of an organic compound is relatively high, it reacts with hydrogen atoms and $OH^{\bullet}$ radicals (and $O^{-\bullet}$) to give reducing radicals able to reduce an additional amount of T^+ to $T^{\bullet}$. The corresponding reactions for $HCOO^-$ taken as an example can be written as follows:

$$H + HCOO^- \xrightarrow{k_{14}} C^{\bullet}OO^{-\bullet} + H_2\,, \qquad (14)$$

$$OH(O^-) + HCOO^- \xrightarrow{k_{15}} COO^{-\bullet} + H_2O(OH^-), \qquad (15)$$

$$T^+ + COO^{-\bullet} \xrightarrow{k_{16}} T^{\bullet} + CO_2\,. \qquad (16)$$

The occurrence of reaction (16) is confirmed by high k_{16} values determined by pulse radiolysis (see Table 3). However, these reactions are not controlled by diffusion; therefore, the k_{16} values correlate with the potentials of the first reduction wave for the corresponding tetrazolium salts measured by polarography.[21]

Hydrogen peroxide reacts with $T^{\bullet}$ [reaction (11)], and the $OH^{\bullet}$ radical formed in this process participates in reaction (16). This yields radical anions $COO^{-\bullet}$, which then reduce T^+. Thus, the yield of formazan can be calculated as follows:

$$G(F) = \frac{1}{2}[G(e_{aq^-}) + G(H) + G(OH)]. \qquad (17)$$

The calculation by this expression using the yields of the water radiolysis products reported in our monograph [26] affords $G(F) = 3.2$ molecule $(100\ eV)^{-1}$. The corresponding experimental results are close to this value (see Table 1).

3. Mechanism of radiolytic reduction. Intermediate products

The properties of tetrazolium radicals $T^{\bullet}$ and the H- and OH-adducts formed by tetrazolium salts in aqueous solutions have been studied by pulse radiolysis.[21, 23, 32, 35] The optical characteristics of $T^{\bullet}$ (see Table 4) were measured in deaerated neutral or alkaline aqueous solutions of TPTC and NPTC containing *tert*-butyl alcohol.[32] In these solutions, $OH^{\bullet}$ radicals react with the alcohol

$$OH^{\bullet} + (CH_3)_3COH \longrightarrow H_2O + \dot{C}H_2(CH_3)_2COH. \qquad (18)$$

The *tert*-butanol radical formed is inert towards tetrazolium salts. *tert*-Butyl alcohol can also react with hydrogen; however, the rate constant for this reaction is relatively low (1.2×10^5 litre $mol^{-1}\ s^{-1}$).[40] Under the conditions employed in the study cited,[32] small amounts of the H-adduct could be formed. The reaction of T^+ with e^-_{aq} in these solutions gives mainly $T^{\bullet}$ radicals. The optical characteristics of the $T^{\bullet}$ radicals were obtained after applying a correction for the absorption of the H-adduct.

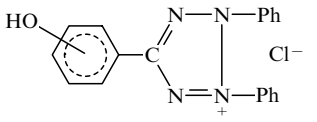
The optical characteristics of the H-adducts $TH^{+\bullet}$ (see Table 4) were determined using deaerated acidic (pH 2) solutions of TPTC and NPTC containing *tert*-butyl alcohol.[32] In these solutions, hydrated electrons react with H^+ [reaction (13)] being thus converted into hydrogen atoms, and the $OH^{\bullet}$ radicals react with the alcohol [reaction (18)] to give $\dot{C}H_2(CH_3)_2COH$ radicals, which are inert with respect to tetrazolium salts. The subsequent transformations afford only the H-adducts in which hydrogen is attached to the benzene ring of the molecule. This conclusion is based on the following observation.[32] Tetrazolium salt molecules contain two types of sites, which could be reactive towards H atoms, namely, the tetrazole heterocycle and the benzene rings. If hydrogen were attached to the tetrazolium ring, the tetrazolium salt would be simultaneously reduced; however, this is not observed experimentally. Formazans are not formed in relatively acidic media (see Fig. 2).

The spectra of the OH-adducts of tetrazolium salts were recorded for deaerated neutral aqueous solutions of TPTC and NPTC saturated with nitrogen hemioxide.[32, 35] In these solutions, e^-_{aq} reacts with N_2O and water giving rise to $OH^{\bullet}$ radicals.

$$e^-_{aq} + N_2O + H_2O \xrightarrow{k_{19}} OH^{\bullet} + N_2 + OH^-. \qquad (19)$$

When this solution is exposed to radiation, OH-adducts and, to a much lesser extent, H-adducts are formed. The optical characteristics of the OH-adducts listed in Table 4 were determined after applying a correction for the absorption of the H-adducts. The rate constants for the reactions of $OH^{\bullet}$ radicals with tetrazolium salts are close in the order of magnitude to the rate constants for the reactions of $OH^{\bullet}$ with benzene derivatives;[40] this confirms the assumption that the $OH^{\bullet}$ groups are attached to a benzene ring in the tetrazolium salt molecule.

The structure of the OH-adduct formed by TPTC has been determined by quantum-chemical calculations.[41] It was found that the $OH^{\bullet}$ radical in the TPTC molecule is bound most likely to the benzene ring that is attached to the carbon atom of the tetrazolium ring. This structure is described by the formula



No formazan is formed in solutions saturated with nitrogen hemioxide;[32] this means that the H- and OH-adducts are not formazan precursors. Stable products formed in these solutions are soluble in water and absorb light in the ultraviolet region of the spectrum (the maxima of the absorption bands are at 300 and ~320 nm for solutions of TPTC and NPTC, respectively).[32]

The tetrazolium radicals and the H-adducts are consumed in second-order reactions (see Table 3). It has been noted above that both in aqueous and in alcoholic solutions, the $T^{\bullet}$ radicals derived from TPTC and NPTC disappear not only through disproportionation but also through dimerisation.

The ratios of the rate constants for disproportionation and dimerisation in neutral aqueous solutions of TPTC or NPTC are equal to 0.5 and 1, respectively (*i.e.* they are much smaller than the corresponding ratio for isopropanol solutions of TPTC; as noted above, the latter is ~1.9).[21] The ratio of these rate constants depends on the pH; it is greater in alkaline solutions than in

neutral ones. This dependence might be due to a change in the structure of the tetrazolium radicals in alkaline media, which decreases the probability of dimerisation because of steric hindrance. When the pH values are sufficiently high, dimerisation does not occur at all.

The OH-adducts disappear in first-order reactions[32] (see Table 3). This might be an intramolecular rearrangement of the OH-adducts or their decomposition with abstraction of a water molecule. Similar processes have been observed for the OH-adducts formed by phenol, aniline and toluene.[26]

4. Radiolysis of aerated solutions

The $G(F)$ values for solutions of TPTC and NPTB saturated with air are substantially smaller than those for deaerated solutions (Table 5). A low yield of formazan [~ 0.16 molecule $(100\ eV)^{-1}$] has also been found[42] for X-ray radiolysis of an aerated 2% aqueous gel of agar-agar containing 0.09 mol litre^{-1} of TPTC (the maximum energy of radiation was 60 keV).

Table 5. Formazan yields [$G(F)$/ molecule $(100\ eV)^{-1}$] upon γ-radiolysis of deaerated and aerated aqueous and water–isopropyl alcohol (1 : 1 by volume) solutions of tetrazolium salts at various pH.[23,24]

Tetrazolium salts	Concentration/ mol litre^{-1}	Presence of alcohol	Gas saturating the solution	pH	$G(F)$
TPTC	8×10^{-4}	yes	argon	11.0	3.1
	8×10^{-4}	"	air	11.0	0.14
	1×10^{-3}	no	argon	6.9	0.45
	1×10^{-3}	"	air	10.3	1.2
	1×10^{-1} (see [a])	"	"	7.0–7.5	0.22
NPTC	1×10^{-3}	"	argon	6.9	0.6
	1×10^{-3}	"	air	6.9	0.45
	1×10^{-3}	"	argon	11.3	1.3
	1×10^{-3}	"	air	11.3	1.5
	4×10^{-3}	yes	"	10.5	4.9
	1×10^{-2}	"	"	10.5	5.3
NPTB	5×10^{-4}	no	"	6.9	0.3
	5×10^{-4}	"	argon	6.9	1.2

[a] Radiolysis under the action of X-rays (the maximum energy 60 keV). The solution contained the triethanolamine–HCl buffer.[22]

However, in the case of aqueous solutions of NPTC, this difference in $G(F)$ is not manifested (see Table 5). This is due to the fact that the tetrazolium radical $TPT^{\bullet}$ generated from TPTC, as in alcoholic solutions, is able to react with oxygen to give the initial TPT^{+} ion [see reaction (9)], whereas NPTC, on the contrary, can be reduced by the $O_2^{-\bullet}$ radical ions[24]

$$NPT^{+} + O_2^{-\bullet} \xrightarrow{k_{20}} NPT^{\bullet} + O_2 . \qquad (20)$$

Therefore, in aqueous and alcoholic solutions of NPTC, when the concentration of the tetrazolium salt is relatively high, atmospheric oxygen hardly influences the yield of formazan. In the case of solutions in aqueous alcohols, for the $R\dot{C}HOH$ radicals to be involved in the interaction with NPTC, the concentration of this salt needs to be high enough. Otherwise some of the alcohol radicals would react with oxygen to give peroxy radicals $RCH(OH)O_2^{\bullet}$, which are apparently unable to reduce NPTC.[24]

The conclusions about the reasons for the low yields of formazan in aerated solutions of TPTC and NPTB have been confirmed by the results of a study carried out by the pulse radiolysis method.[23] It was found that the tetrazolium radicals $T^{\bullet}$ formed in these solutions in the presence of *tert*-butyl alcohol disappear much faster than in deaerated solutions. The constants k_9 were determined by measuring the kinetics of consumption of $T^{\bullet}$ in the presence of oxygen (see Table 3).

The direction of electron transfer in reactions the rates of which are not controlled by diffusion may be determined, for instance, by the electron affinity of the reactants. Evidently, the electron affinity of the oxygen molecule should be higher than that of the TPTC and NPTB molecules but lower than that of the NPTC molecule.[23] This is confirmed by the results of determination of the half-wave potentials for the polarographic reduction of tetrazolium salts.[21] The potentials for the single-electron reduction of tetrazolium salts and two-electron reduction of oxygen are -0.40, -0.30, -0.17, and -0.30 V for TPTC, NPTB, NPTC, and O_2, respectively.[21,23]

The $G(F)$ values attained at high concentrations of NPTC are larger than the theoretical value (see Table 5) calculated from Eqn (17) using the known yields of the products of water radiolysis. It cannot be ruled out that in this system, due to the relatively high concentrations of conjugated acceptors (the alcohol, on the one hand, and TPTC and oxygen, on the other hand), the reactions in spurs are largely suppressed, and this results in increased yields of the primary radiolysis products, responsible for the formation of formazan.

5. Radiolytic reduction of Nitrotetrazolium Blue

Interesting results have been obtained in a pulse radiolysis study of the mechanism of reduction of Nitrotetrazolium Blue in aqueous solutions.[10,33] The reduction of the two-charged cation, NTB^{2+}, contained in this molecule to give the corresponding diformazan is a four-electron process, which gives consecutively the monotetrazolium radical $NTB^{+\bullet}$, monoformazan MF^{+}, monoformazan radical $MF^{\bullet}$ and finally diformazan DF.

Conditions were selected[33] under which exposure of a formate-containing aqueous solution of NTB^{2+} to an electron pulse affords $NTB^{+\bullet}$. The rate constant for the reduction of NTB^{2+} with the $COO^{-\bullet}$ radical anion was measured (see Table 3). Study of the kinetics of consumption of $NTB^{+\bullet}$ showed that the rate constant for this process depends crucially on the pH. The following reaction was postulated for a neutral medium:

$$2NTB^{+\bullet} + H_2O \xrightarrow{k_{21}} NTB^{2+} + MF^{+}...OH^{-} . \qquad (21)$$

The reaction in an alkaline medium yields the hydroxy-derivative $NTB^{+\bullet}...OH^{-}$, which disappears more rapidly than $NTB^{+\bullet}$. The corresponding reaction can be written as follows:

$$2(NTB^{+\bullet}...OH^{-}) + H_2O \xrightarrow{k_{22}} NTB^{2+} + MF^{+}...OH^{-} + 2OH^{-} . \qquad (22)$$

The k_{21} and k_{22} values found for these reactions are listed in Table 3. The higher rate of reaction (22) is apparently due to the fact that the positive charge of $NTB^{+\bullet}$ is shielded by the hydroxyl ion. Dimerisation of $NTB^{+\bullet}$ does not occur, probably for steric reasons.

The kinetics of the reaction

$$NTB^{2+} + O_2^{-\bullet} \xrightarrow{k_{23}} NTB^{+\bullet} + O_2 \qquad (23)$$

were studied by the stopped flow method.[33]

The constant k_{23} found in this study is presented in Table 3. It was noted that the back reaction does not occur under the conditions employed. It was concluded that Nitrotetrazolium Blue can be used for qualtitative but not quantitative determination of the $O_2^{-\bullet}$ radical anions. It should also be emphasised that H_2O does not react with NTB^{2+}.

IV. Solid matrices

Polymeric films with tetrazolium salts (mainly TPTC) introduced into them have been mostly studied as applied to the dosimetry of ionising radiation (see above). Radiolytic transformations of

TPTC and NPTC in poly(vinyl alcohol) films have been studied in detail.[25]

The paramagnetic species formed in poly(vinyl alcohol) films irradiated under various conditions have been investigated.[25,43,49] The yield of these species is ~ 4 molecule $(100\ eV)^{-1}$. The EPR spectrum of an irradiated film recorded at 77 K points to the presence of three paramagnetic species: a captured electron and the $\sim CH_2CH[OC^{\bullet}(CH_3)O^-H^+]CH_2\sim$ and $\sim CH_2C^{\bullet}(OH)CH_2\sim$ radicals. At room temperature, only the former radical is detected.

All the paramagnetic species mentioned above possess reducing properties. At relatively high temperatures, the species become mobile, and their interaction with TPTC and NPTC resulting in the formation of formazans becomes possible. This conclusion is confirmed experimentally. Table 1 presents the G(F) values obtained at room temperature for films containing various amounts of these salts.[25] It is noteworthy that the formazan concentration obeys a linear dependence on the radiation dose up to a dose of 25 kGy.

Evidently, the formation of formazan in films is also a two-electron process, T^+ ions being reduced in reactions with mobile electrons and with the radicals mentioned above. For example, the following reaction is possible [25]

$$T^+ + \sim CH_2\underset{\displaystyle OH}{\overset{\displaystyle |}{\dot{C}}}CH_2\sim \longrightarrow T^{\bullet} + \sim CH_2\underset{\displaystyle O}{\overset{\displaystyle ||}{C}}CH_2\sim + H^+. \qquad (24)$$

Thus, G(F) should be $0.5\,G$(radical),† *i.e.* it should be equal to ~ 2 molecule $(100\ eV)^{-1}$. However, only in a film containing 7.1×10^{-3} mol litre^{-1} of TPTC, $G(F) < 0.5\,G$(radical); in all other cases, G(F) is greater than $0.5\,G$(radical) (see Table 1), and for large concentrations of NPTC it is more than twice as great. It can be concluded that G(F) is determined both by the distance between tetrazolium salt molecules and by the electron-acceptor properties of these molecules.[25]

A similar situation has been observed for poly(vinyl alcohol) films containing methylviologen MV^{2+}.[47,48] When these films are exposed to radiation, the colourless MV^{2+} dication is reduced *via* a single-electron process to give $MV^{+\bullet}$, which has an intense blue colour. According to several measurements,[44,45] the $G(MV^{+\bullet})$ values vary from 8.1 to 9.8 molecule $(100\ eV)^{-1}$. This is much larger than the G(radical) value.

It was postulated [25] that the high G(F) values observed are due to the reactions of T^+ with radical pairs $R_1^{\bullet}...R_2^{\bullet}$, which are formed in some polymers including poly(vinyl alcohol) films (see, for example, Ref. 50)

$$T^+ + R_1^{\bullet}...R_2^{\bullet} \longrightarrow R_1^{\bullet} + R_2^+ + T^{\bullet}. \qquad (25)$$

In the absence of tetrazolium salts, the radical pair undergoes recombination. The EPR spectrum of a poly(vinyl alcohol) film contains an unresolved signal with a g-factor of 4 corresponding to this radical pair;[25] the intensity of this signal is four orders of magnitude lower than that of the signal characterised by a g-factor of 2. Another possible reason is the transfer of the excitation energy along the chain of polymer bonds to a tetrazolium salt molecule.

Thus, in a solid matrix of a polymeric alcohol, tetrazolium salts are also reduced to formazans by electrons and 'alcohol' radicals.

V. The use of tetrazolium salts in dosimetry

The property of colourless or yellowish liquid or solid solutions of tetrazolium salts to turn pink, orange, or orange-red upon radiolytic reduction to formazans has been recommended for use in the dosimetry of ionising radiations.

1. Alcoholic, aqueous and water – alcohol solutions

Back in the late 1940s,[12] it was found that aqueous solutions and gelatine emulsions of TPTC change to a red colour on exposure to UV light ($\lambda < 365$ nm), α-particles, or X-rays. It was also found that the colour intensity did not depend on the pH in the range 5.5 – 6.6

In a number of publications (see, for example, Refs 22, 42, 51 – 53), it has been suggested to use TPTC in radiology and radiobiology in order to obtain three-dimensional images of dose distribution upon X-ray treatment. For example, a 2% aqueous gel of agar-agar containing TPTC (0.05%) has been used [52] to model the three-dimensional dose distribution in fish and meat foodstuffs treated with X-rays (maximum energy 60 keV, dose ~ 15 kGy). It has been reported [42] that gels of this type are suitable for measurement of three-dimensional dose distribution for X-ray and electron radiations. It was also shown that the concentration of the formazan produced depends linearly on the radiation dose up to a dose of 12 MGy.

The influence of various parameters (the nature of the buffer solution, TPTC concentration, the presence of oxygen in the solution, and the dose rate) on the yield of formazan has been studied in detail.[22] The precipitate of formazan formed upon irradiation with X-rays with a maximum energy of 60 keV was dissolved in an organic solvent (for example, in tetrahydrofuran) added to the solution. It was found that in aerated solutions, G(F) does not depend on the pH of the solution over the range 5 – 8. At higher pH values the yield increases, while at lower pH it decreases, and becomes equal to zero at pH 1.5. If the concentration of TPTC increases, the yield first increases and then remains constant in the concentration range from 3×10^{-3} to 1×10^{-1} mol litre^{-1}. The G(F) values in deaerated solutions are approximately twice as large as those in aerated solutions. In systems saturated with oxygen, the G(F) values are 2.7 times lower than in aerated solutions. The addition of hydrogen peroxide decreases G(F). It was proposed to use aerated 2×10^{-2} – 1×10^{-1} M aqueous solutions of TPTC containing a triethanolamine – HCl buffer for the measurement of absorbed X-ray radiation doses ranging from 0.18 kGy to 0.18 MGy. Note that the dosimetric solution should be stored in the dark.

The possibility of using aerated ethanolic solutions of TPTC for determination of the absorbed doses of γ-radiation has been studied.[37] Solutions in ethanol containing 5% of methanol and 5% of isopropanol were used. It was found that a 4.5×10^{-2} M solution of TPTC can be used to measure radiation doses in the range 1 – 16 kGy. For lower concentrations of TPTC, the upper limit of the measurable doses decreases (at higher doses, the dependence of the formazan concentration on the dose becomes nonlinear, because the formazan starts to decompose).

Figure 3 presents the dependence of the optical absorption of aerated solutions of TPTC on the dose. It can be seen that the yield of formazan diminishes with a decrease in the TPTC concentration; the yield obtained in a 5×10^{-3} M solution is ~ 2.3 times

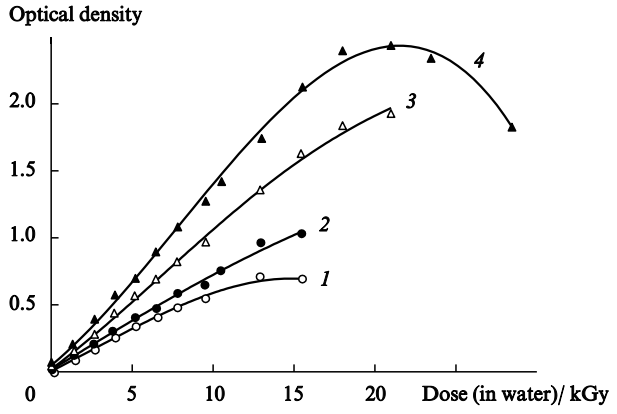


Figure 3. Optical density ($\lambda = 480$ nm) of aerated 5×10^{-3} (*1*), 1×10^{-2} (*2*), 1.8×10^{-2} (*3*), and 4.5×10^{-2} M (*4*) ethanolic solutions of TPTC versus the radiation dose (optical path length 0.1 cm).

† G(radical) implies the yields of both radicals and electrons.

lower than that in a 4.5×10^{-2} M solution. The yields also depend on the temperature. The temperature coefficient in the range 0–30 °C is approximately +0.8% per 1 °C, but in the temperature range between 30 and 60 °C, it is substantially larger. Non-irradiated and radiated solutions are stable during storage in the dark in sealed tubes for at least 5 days. However, exposure to daylight and especially to bright sunlight leads to slow formation of formazan upon photolysis. If the solution is saturated with oxygen, the upper limit of determination of the dose increases to 36 kGy. Deaerated solutions are more 'sensitive' but the upper limit decreases to ~3 kGy. The optical characteristics of 1,3,5-triphenylformazan and other formazans are listed in Table 6.

Table 6. Optical characteristics of formazans.

Formazan	Matrix	λ_{max}/ nm	$10^{-3}\,\varepsilon_{max}$ / $m^2\,mol^{-1}$	Ref.
1,3,5-Triphenyl-formazan	ethanol [a]	480	1.5	39
	isopropanol	485	1.6	18
	acetone	480	–	42
	water–isopropanol mixture (1 : 1 by volume)	485	1.6	21, 24
	2% aqueous agar-agar gel	515, 565	–	42
	poly(vinyl alcohol)	500	0.92	25
	the same	493, 540 (shoulder) [b]	–	35
	"	490	–	55
	polyvinyl-pyrrolidone	550 [c]	–	56
1-(*p*-Nitrophenyl)-3,5-diphenylformazan	water–isopropanol mixture (1 : 1 by volume)	475–480	1.9	21, 24
	polyvinyl-pyrrolidone	500	0.80	25
1,5-Diphenyl-3-(*p*-nitrophenyl)formazan	water–isopropanol mixture (1 : 1 by volume)	480	1.2	21
Monoformazan from NTB	water, pH 5.7–6.7	530	1.28	33
	water, pH 9.5–11.0	530	2.54	33
Monoformazan from TB	aqueous ethanol	560	–	54
	poly(vinyl alcohol)	540	–	54

[a] Ethanol contained 5% of methanol and 5% of isopropyl alcohol. [b] The shoulder at 540 nm belongs apparently to ditetrazolium salt. [c] The band at 550 nm might belong to ditetrazolium salt.

The formation of formazan in solutions of TPTC is inhibited by oxygen. Figure 4 shows the concentrations of the formazan formed in aerated 1×10^{-3} M aqueous solutions of TPTC *versus* the γ-radiation dose for two different pH values.[24] It can be seen that at low doses almost no formazan is formed. When the doses are larger than 0.8 kGy and oxygen in the solution is consumed almost entirely, the yield of formazan increases substantially.

A similar type of behaviour has been observed in the case of aerated solutions of Tetrazolium Blue in aqueous alcohols. These properties have been used to develop a threshold chemical dosimeter.[54] The threshold dose is detected by a sharp increase in the optical absorption of the solution in the maximum of the formazan band. This dose changes upon variation of the concentration of ethanol (the solubility of air in ethanol is approximately an order of magnitude higher than that in water) or tetrazolium

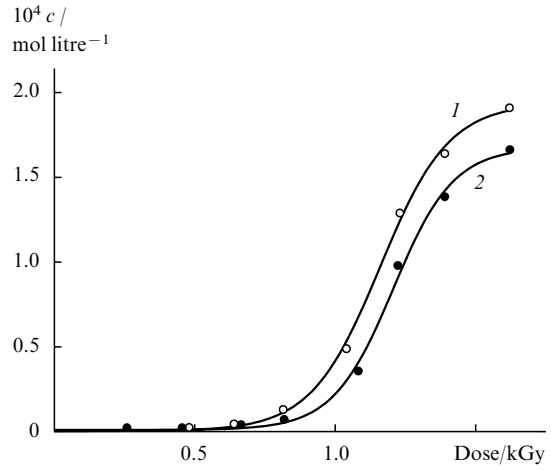


Figure 4. Dependence of the formazan concentration in an aerated 1×10^{-3} M aqueous solution of TPTC at pH 10.3 (*1*) and 8.5 (*2*) on the absorbed dose.

salt in the solution. The threshold doses measured by means of aerated solutions of TPTC or TB in aqueous ethanol amount to 1.6–3.4 kGy.

It follows from the foregoing that, due to the 'inhibiting' effect of oxygen, aerated aqueous and alcoholic solutions of TPTC can be used only for determination of relatively large radiation doses. It has been found [24] that this effect is not manifested in aqueous solutions of NPTC (see Table 5). An aerated $(0.4–1) \times 10^{-2}$ M solution of NPTC in a water–isopropyl alcohol mixture (1 : 1 by volume) containing 0.2 mol litre^{-1} of formate (pH 9.5–11) can be used to determine absorbed γ-radiation doses in the range between 5 and 50 Gy (Fig. 5). Formate is needed to eliminate the short 'induction period'. The sensitivity of the spectrophotometric method for formazan analysis based on the use of a system with a 5×10^{-3} M solution of NPTC amounts to 6×10^{-3} Gy^{-1} cm^{-1}. When the NPTC concentration is equal to 1×10^{-2} mol litre^{-1}, the sensitivity of the method increases to 1×10^{-2} Gy^{-1} cm^{-1}, and the threshold of the dose determination decreases to 1 Gy.

The optical absorption of irradiated solutions changes by not more than 3% over a period of 24 h. The solutions are sensitive to

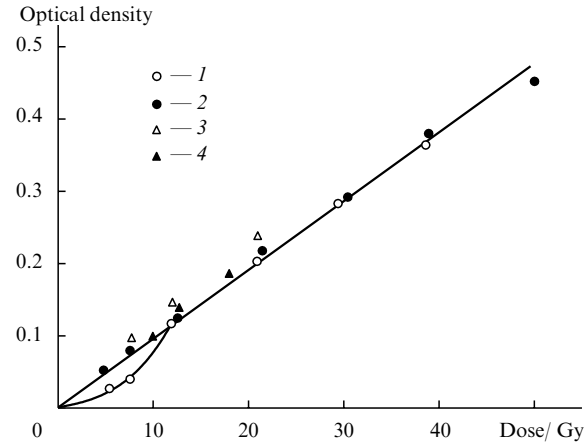


Figure 5. Dependence of the optical density (λ = 475 nm) of aerated water–alcohol (1 : 1 by volume) solutions of NPTC on the γ-radiation dose;
(*1*) a 4×10^{-3} M water–isopropanol solution, pH 10.5; (*2*) the same solution containing 0.2 mol litre^{-1} of formate; (*3*) a 1×10^{-3} M water–isopropanol solution containing 0.2 mol litre^{-1} of formate, pH 10.5; (*4*) a 4×10^{-3} M water–ethanol solution containing 0.2 mol litre^{-1} of formate, pH 9.5 (optical path length 1 cm).

light; therefore, they should be stored in the dark. The results are liable to a positive temperature effect, but if the measurements are carried out at 20 ± 5 °C, this effect can be neglected.

Similar results have been obtained with aerated weakly alkaline (pH 8.5–9) solutions of NTB (concentration $1.5 \times 10^{-4} - 4.5 \times 10^{-3}$ mol litre^{-1}) in aqueous alcohols (1 : 1 by volume).[54] Aqueous solutions of ethanol and isopropanol were used. This method permits determination of doses from 5 to 150 kGy.

2. Polymer films

Dosimetric systems based on tetrazolium salts in polymeric films have been described in the literature. The Kodak Pathe company (France) have developed a dosimetric gelatine film containing TPTC, suitable for measuring doses of about tens of kilogray.[57] Practical details of the application of these films in the dosimetry of ionising radiation can be found in the literature.[55, 58, 59]

Other TPTC-containing polymeric films, for example, poly(vinyl acetate),[37] polyvinylpyrrolidone,[56] and poly(vinyl alcohol) films [25, 35, 54, 60] have also been proposed for dosimetry.

Poly(vinyl alcohol) films have been studied most comprehensively. These films can be used to measure absorbed doses in the range from 0.5 to several tens of kilogray.[25] Another publication [35] presents a somewhat different range of measurable doses, from 1 to 100 kGy. For NPTC-containing films, the lower limit of determination of absorbed doses is 0.1 kGy.[25] In the case of films with TPTC, the readings do not depend on the dose rate, at least in the range from 0.96 to 3.2×10^7 Gy s^{-1}.[35, 64] The optical absorption of irradiated films containing TPTC or NPTC changes by not more than 14% over a period of 27 days.[54] However, the readings are somewhat dependent on the temperature. The temperature coefficient for the readings for TPTC-containing poly(vinyl alcohol) films is about $+0.46\%$ per 1 °C. This can be neglected if the measurements are performed at 20 ± 5 °C. The main drawback of films containing monotetrazolium salts is that they are sensitive to light. Therefore, they should be stored in the dark. This drawback can be largely eliminated if TB is used as the radiochromic additive.[54] During storage under ordinary conditions, the optical absorption of these films remains almost invariable over a period of 14 days. Transparent and matt films containing TB have been used for a long period as dose indicators for radiation sterilisation of medical wares.[60] The use of matt films is due to the need to distinguish more clearly the colours of nonirradiated and irradiated films.

It has been proposed [35] that TPTC-containing poly(vinyl acetate) films can serve for determination of doses between 5 and 50 kGy. In addition, they can be used to obtain radiographic images. It has been suggested [56] that polyvinylpyrrolidone films with TPTC may find application for determination of doses of up to 40 kGy, while NTB can be used as a radiochromic additive to polymeric matrices.

VI. Conclusion

The above discussion leads to the following conclusions.

1. The use of radiation-chemical methods (first of all, pulse radiolysis) made it possible to study the kinetics of reduction of tetrazolium salts to formazans in aqueous and alcoholic solutions and in solid matrices and to elucidate the nature of the intermediate species.

2. The investigation of radiolytic transformations of tetrazolium salts provided more precise data about some aspects of the action of ionising radiation on liquid alcohols and poly(vinyl alcohol) films. In particular, the nature of the free radicals formed in irradiated poly(vinyl alcohol) was determined, and the hypothesis of participation of radical pairs in radiation-induced chemical reactions with admixtures present in the polymer was confirmed.

3. Studies of the radiolytic transformations of tetrazolium salts led to new applications of these compounds in chemistry and biochemistry.

4. The development of dosimetric systems based on tetrazolium salts suitable for the measurement of different ranges of absorbed doses and their three-dimensional distribution in irradiated objects is fairly significant from the practical viewpoint.

The main drawback of these systems (their low resistance to light), which holds up their wide use, can be largely eliminated by using poly(vinyl alcohol) films containing Tetrazolium Blue as a radiochromic additive.

References

1. H von Pechmann, P Runge *Berichte* **27** 2920 (1894)
2. G Lakon *Ber. Deut. Botan. Ges.* **60** 299 (1943)
3. H J Cottrell *Nature* (*London*) **159** 748 (1947)
4. A M Mattson, C O Jensen, R A Dutcher *Science* **106** 194 (1947)
5. T D Waugh *Trans. Faraday Soc.* **33** 314 (1948)
6. F H Straus, N D Cheronis, E Straus *Science* **108** 113 (1948)
7. E Farber, E Bueding *J. Histochem. Cytochem.* **4** 357 (1956)
8. F P Altman *Prog. Histochem. Cytochem.* **9** 51 (1976)
9. D Amano, Y Kagosaki, T Usui, S Yamamoto, O Hayaishi *Biochem. Biophys. Res. Commun.* **66** 272 (1975)
10. B H J Bielski, H W Richter *J. Am. Chem. Soc.* **99** 3019 (1977)
11. C Auclair, M Torres, J Hakim *FEBS Lett.* **89** 26 (1978)
12. Z S Gierlach, A T Krebs *Am. J. Roentgenol. Radium Therapy* **62** 559 (1949)
13. A W Nineham *Chem. Rev.* **55** 355 (1955)
14. O W Maender, G A Russell *J. Org. Chem.* **31** 442 (1966)
15. F A Neugebauer, G A Russell *J. Org. Chem.* **33** 2744 (1968)
16. I S Trubnikov *Zh. Org. Khim.* **12** 1373 (1976) [a]
17. G G Glenner, in *H J Coon's Biological Stains* Ch. 9 (Ed. R D Lillie) (St.Louis: Sigma Chemical, 1990)
18. Z K Kriminskaya, K M Dyumaev, G V Fomin, A K Pikaev *Khim. Vys. Energ.* **17** 304 (1983) [b]
19. Z K Kriminskaya, N P Makshanova, K M Dyumaev, A K Pikaev *Khim. Vys. Energ.* **21** 501 (1987) [b]
20. Z K Kriminskaya *Khim. Vys. Energ.* **30** 391 (1996) [b]
21. Z K Kriminskaya, A A Konarev, K M Dyumaev, B V Tolkachev, A K Pikaev *Izv. Akad. Nauk SSSR, Ser. Khim.* 1746 (1988) [c]
22. E Winter *Z. Phys. Chem.* **40** 109 (1964)
23. Z K Kriminskaya, A A Konarev, K M Dyumaev, A K Pikaev *Khim. Vys. Energ.* **23** 467 (1989) [b]
24. A K Pikaev, Z K Kriminskaya *Mendeleev Commun.* 200 (1995)
25. Z K Kriminskaya, S F Ginzburg, A A Molin *Khim. Vys. Energ.* **25** 335 (1991) [b]
26. A K Pikaev *Sovremennaya Radiatsionnaya Khimiya. Radioliz Gazov i Zhidkostei* (Modern Radiation Chemistry. Radiolysis of Gases and Liquids) (Moscow: Nauka, 1986)
27. Z K Kriminskaya, K M Dyumaev, A K Pikaev *Izv. Akad. Nauk SSSR, Ser. Khim.* 48 (1985) [c]
28. Z K Kriminskaya, K M Dyumaev, L S Vyshchipanova, Yu V Ivanov, A K Pikaev *Khim. Vys. Energ.* **18** 40 (1984) [b]
29. Z K Kriminskaya, K M Dyumaev, A K Pikaev *Khim. Vys. Energ.* **22** 276 (1988) [b]
30. Z K Kriminskaya, A G Kotov, Yu V Ivanov, N P Kon'kova, B V Tolkachev *Khim. Vys. Energ.* **21** 316 (1987) [b]
31. Z K Kriminskaya, A G Kotov, N P Kon'kova, Yu V Ivanov *Khim. Vys. Energ.* **25** 144 (1991) [b]
32. Z K Kriminskaya, K M Dyumaev, L S Vyshchipanova, Yu V Ivanov, A K Pikaev *Khim. Vys. Energ.* **22** 13 (1988) [b]
33. B H J Bielski, G G Shiue, S Bajuk *J. Phys. Chem.* **84** 830 (1980)
34. A K Pikaev, Z K Kriminskaya, in *The 10th International Meeting on Radiation Processing* (*Conference Program and Abstracts*), *Anaheim, 1997* p. 197
35. A Kovacs, L Wojnarovits, W L McLaughlin, S E E Eid, A Miller *Radiat. Phys. Chem.* **47** 483 (1996)
36. H Zollinger *Colour Chemistry. Syntheses, Properties and Applications of Organic Dyes and Pigments* (Weinheim: Verlagsgesellschaft, 1972)
37. L M Dorfman, N E Shank, S Arai *Advan. Chem. Ser.* (82) 58 (1968)
38. A V El'tsov, O P Studzinskii, V M Grebenkina *Usp. Khim.* **46** 185 (1977) [*Russ. Chem. Rev.* **46** 93 (1977)]

39. A Kovacs, L Wojnarovits, N B El-Assy, H Y Afeefy, M Al-Sheikhly, M L Walker, W L McLaughlin *Radiat. Phys. Chem.* **46** 1217 (1995)
40. A K Pikaev, S A Kabakchi *Reaktsionnaya Sposobnost' Pervichnykh Produktov Radioliza Vody. Spravochnik* (Reactivity of Primary Products of Water Radiolysis. Handbook) (Moscow: Energoizdat, 1982)
41. Z K Kriminskaya, K A Kon'kov *Khim. Vys. Energ.* **26** 111 (1992) [b]
42. Th Gruenewald, W Schmidt-Lorenz *Atomkernenergie* **9** 142 (1964)
43. H Hase, H Yamaoka *Radiat. Eff.* **19** 195 (1973)
44. V S Chervonenko *Khim. Vys. Energ.* **10** 458 (1976) [b]
45. L T Patterson, R D Small, J C Scaniano *Radiat. Res.* **72** 218 (1977)
46. I P Shelukhov, G S Zhdanov, V K Milinchuk *Khim. Vys. Energ.* **15** 324 (1981) [b]
47. T Ogawa, H Nishikawa, S-I Nishimoto, T Kagiya *Radiat. Phys. Chem.* **29** 353 (1987)
48. S-I Nishimoto, M Ye, Y Lu, T Kawamura, T Kagiya *Radiat. Phys. Chem.* **32** 727 (1988)
49. Z K Kriminskaya *Khim. Vys. Energ.* **27** 37 (1993) [b]
50. M Iwasaki, T Ishikawa, T Ohmori *J. Chem. Phys.* **50** 1984 (1969)
51. J G Kereiakes, A T Krebs, in *Medical Physics* Vol. 3 (Ed. O Glasser) (Chicago: The Year Book Publishers, 1960) p. 509
52. W Schmidt-Lorenz, A Berger *Int. J. Appl. Radiat. Isot.* **11** 161 (1961)
53. A T Krebs, in *The Encyclopedia of X-Rays and Gamma Rays* (Ed. G L Clark) (New York: Reinhold Publishing, 1963) p. 274
54. A K Pikaev, Z K Kriminskaya *Radiat. Phys. Chem.* **52** 555(1998)
55. W L McLaughlin, in *Sterilisation by Ionising Radiation* Vol. 1 (Eds E R L Gaughran, A J Goudie) (Montreal: Multiscience Publications, 1974) p. 219
56. W L McLaughlin, in *High Dose Dosimetry for Radiation Processing* (Vienna: IAEA, 1991) p. 3
57. Fr. P. 2 050 221; *Chem. Abstr.* **76** 67 183 (1972)
58. F Bredoux *Societe Kodak Pathe Report, Paris 1972*
59. E T Snow *Report SLA-73-0991, Sandia Laboratories, New Mexico, USA, 1974*
60. RF P. 1 739 766; *Byull. Izobret.* (1) 196 (1994)

[a] *Russ. J. Org. Chem. (Engl. Transl.)*
[b] *High Energy Chem. (Engl. Transl.)*
[c] *Russ. Chem. Bull. (Engl. Transl.)*