The Role of Singlet Oxygen in the Lyoluminescence of Saccharides

E. Pitt, A. Scharmann, and T. Suprihadi *

I. Physikalisches Institut, Heinrich-Buff-Ring 16, W-6300 Gießen, FRG

Z. Naturforsch. **47a**, 463–468 (1992); received December 6, 1991

Lyoluminescence, the radiation induced light emission during the dissolution of irradiated solid substances like alkali halides, saccharides, amino acids etc. was proposed as a method of dosimetry. It is established that lyoluminescence is a consequence of a reaction of radicals from radiolysis with oxygen dissolved in the solvent. The role of oxygen in the lyoluminescence of saccharides has been investigated. It has been proved that oxygen is not only necessary for the chemical reaction preceeding the lyoluminescence process, but that it is also the emission centre itself. Singlet oxygen is released from the disproportionation of peroxyl radicals produced during solution. The lyoluminescence emission peaks may be attributed to the simultaneous transitions $[^1\Sigma_g^+][^1\Sigma_g^+]$, $[^1\Delta_g][^1\Delta_g]$ and $[^1\Sigma_g^+][^1\Delta_g]$ to $[^3\Sigma_g^-][^3\Sigma_g^-]$ of collisional pairs of singlet oxygen molecules.

1. Introduction

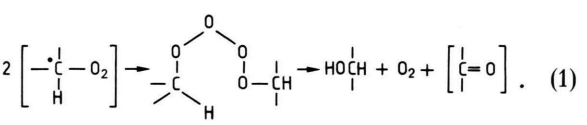
A large number of organic solids exhibit chemiluminescence when dissolved in suitable liquids after exposure to ionizing radiation, the so-called lyoluminescence (LL). Especially saccharides were proposed as personal dosemeter materials because of their tissue-equivalence. But a lower detection limit of 1 Gy-γ-rays has excluded LL from application in routine. Recent investigations have shown that this limit is caused essentially by environmental influences like the temperature and pH of the solvent and by oxygen present during the solution [1]. Controlling the oxygen content has reduced the statistic error of LL measurements. A better understanding of the basic mechanisms running during the light emission should render a lower detection limit. Therefore the role of oxygen during this process has been investigated in this work.

2. Light Emission Processes

a) Concept of Lyoluminescence Emission in Organic Compounds

A radical mechanism was ascertained as the base of lyoluminescence [2], free trapped radicals being formed as a result of exposure to ionizing radiation. Referring to Vassil'ev's [3] model for the chemiluminescence of hydrocarbons, Ettinger and Puite [4] proposed the following description for the mechanism of the LL-process: Radicals are formed in the solid by radiation absorption and oxidized to peroxyl radicals during dissolution. By disproportionation these peroxyl radicals yield a carbonyl compound, an oxygen molecule, and an alcohol fragment. In agreement with oxygen consumption during dissolution of irradiated saccharides in water [5], Baugh et al. showed that the LL-intensity depends on dissolved oxygen in the solvent [6]. In an earlier investigation [1] we could give a quantitative description of the lyoluminescence output as a function of oxygen concentration in water and of the absorbed dose. The oxygen consumption by the lyoluminescence system is proportional to its initial concentration. This is a proof for the assumption of oxydation in the lyoluminescence process.

Ettinger and Puite [4] explained lyoluminescence in Russel's [7] model. This model deals in general with the disproportionation of a peroxyl radical and the distribution of its excitation energy to the resulting molecules. Russel proposed that two secondary peroxyl radicals undergo a self-reaction generating a metastable cyclic transition state:



(1)

In its disproportionation there are two possibilities for the reaction products in consideration of the spin

* On leave from Indonesian National Atomic Energy Agency, (BATAN). Centre for Standardization and Radiological Safety Research (PSPKR) P.O. Box 43 KBYL, Jakarta.

Reprint requests to Prof. Dr. A. Scharmann, I. Physikalisches Institut, Universität Gießen, Heinrich-Buff-Ring 16, W-6300 Gießen, FRG.

0932-0784 / 92 / 0300-0463 $ 01.30/0. – Please order a reprint rather than making your own copy.

multiplicity an energy state, respectively, of the oxygen molecules:

1. Oxygen in the triplet groundstate and the accompanying carbonyl compound in the triplet excited state, or
2. oxygen in the singlet excited state ($^1\Sigma$ or $^1\Delta$) and the accompanying carbonyl compound in the singlet groundstate.

Ettinger et al. [8] proposed the carbonyl compound as an emission centre of LL. They apply Vassil'ev's suggestion for chemiluminescence reactions of hydrocarbons. These molecules emit a broad band in the range 420–490 nm, which has been observed as well during the relaxation of ethylmethyl ketone. In ethylmethyl ketone the emission was attributed to its carbonyl binding.

The LL of amino acids exhibits a similar single broad emission band in the range 450–490 nm [4, 9], so the LL is described with the relaxation of an excited carbonyl compound. The LL of saccharides, in contrast, consist of a spectrum composed by several peaks and in general stretches towards longer wavelengths, well beyond 600 nm.

With respect to this fact, Ettinger and Puite [4] discussed the LL-spectra of saccharides as an emission of a relaxing oxygen molecule complex $(^1O_2^*)_2$. They suggested a so-called collisional pair of singlet oxygen molecules $^1O_2^* - ^1O_2^*$ as an emission centre. It is generated by the summary reaction

$$RO_2^{\cdot} + RO_2^{\cdot} \rightarrow R-R + (^1O_2^*)_2 . \qquad (2)$$

It is consistent with singlet oxygen production in Russel's model and the transfer of the excitation energy to the oxygen molecule. It is supported by the fact that the LL-intensity of saccharides increases with increasing oxygen concentration of solvent in the range 1.5–15 mg/l O_2 [1]. The LL of amino acids, in contrast, decreases with increasing oxygen concentration [4]. Therefore the LL of amino acids cannot be described in the same way. It is known that the weak intensity of chemiluminescence reactions of hydrocarbons can be explained by quenching of excited carbonyl bindings by groundstate triplet oxygen [10]. The same effect may explain the oxygen dependence of the LL of amino acids.

b) Singlet Oxygen

The absorption and emission spectra of singlet oxygen were investigated and discussed in detail [11]. The groundstate configuration of molecular oxygen is

$$KK(\sigma_g 2s)^2(\sigma_u 2s)^2(\sigma_g 2p)^2(\pi_u 2p)^4(\pi_g 2p)^2 .$$

It can give rise to the three states: $^3\Sigma_g^-$ (ground state), $^1\Delta_g$, and $^1\Sigma_g^+$.

In a free oxygen molecule, the following electronic transitions can be observed both in absorption and in emission:

$$^1\Delta_g \rightleftharpoons {}^3\Sigma_g^-, \quad \Delta E = 1270 \text{ nm},$$
$$^1\Sigma_g^+ \rightleftharpoons {}^3\Sigma_g^-, \quad \Delta E = 730 \text{ nm}.$$

They are strictly electric dipole forbidden. The life times are 45 min for $^1\Delta$ and about 7 sec for $^1\Sigma$. In the condensed phase they are shortened by molecular collisions ($\tau_{^1\Sigma} \simeq 10^{-9}$ s and $\tau_{^1\Delta} \simeq 10^{-3}$ s). As a consequence, the emission of these two transitions vanishes. But additional bands are observed in the visible. They correspond to various combinations of pairs of single molecule transitions. The corresponding wavelengths are [12–17]

$$^1\Sigma_g^+ \, {}^1\Sigma_g^+ (0,0) \rightarrow {}^3\Sigma_g^- \, {}^3\Sigma_g^- (0,0) \quad 380–400 \text{ nm},$$
$$^1\Delta_g^+ \, {}^1\Sigma_g^+ (0,0) \rightarrow {}^3\Sigma_g^- \, {}^3\Sigma_g^- (0,0) \quad 480 \text{ nm},$$
$$^1\Delta_g^+ \, {}^1\Delta_g^+ (2,0) \rightarrow {}^3\Sigma_g^- \, {}^3\Sigma_g^- (0,0) \quad 520 \text{ nm},$$
$$^1\Delta_g^+ \, {}^1\Delta_g^+ (1,0) \rightarrow {}^3\Sigma_g^- \, {}^3\Sigma_g^- (0,0) \quad 580 \text{ nm},$$
$$^1\Delta_g^+ \, {}^1\Delta_g^+ (0,0) \rightarrow {}^3\Sigma_g^- \, {}^3\Sigma_g^- (0,0) \quad 630 \text{ nm},$$
$$^1\Delta_g^+ \, {}^1\Delta_g^+ (0,1) \rightarrow {}^3\Sigma_g^- \, {}^3\Sigma_g^- (0,.0) \quad 700 \text{ nm}.$$

3. Methods and Measurements

The role of singlet oxygen in lyoluminescence of saccharides was investigated in three step:

1. Test of the presence of singlet oxygen during the emission,
2. Influence of reduction of singlet oxygen concentration on LL,
3. Investigation of LL-spectra.

Mannose and fructose were chosen as objects. The integral LL output was measured with a chemiluminescence analyzer LB 950 (Berthold [1]). The solvent was injected, controlled by a computer. The LL-pulse was integrated for 10 s.

3.1. Test of the Presence of Singlet Oxygen

Singlet oxygen can be indentified by its reaction with a selective oxygen acceptor. 1,3-diphenylisobenzo-

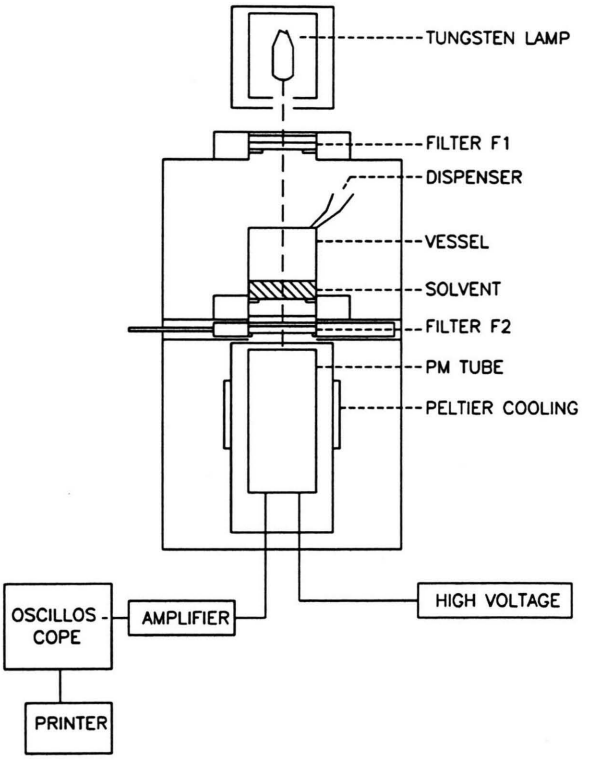


Fig. 1. Optical set-up for the registration of singlet oxygen dissappearance by DPBF addition during LL.

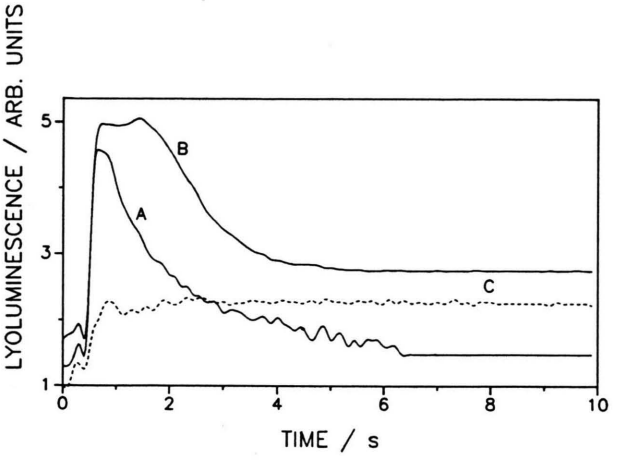


Fig. 2. LL-pulse of mannose (100 Gy) in water (A) and water/DPBF-aceton (B). Phosphorescence of DPBF in aceton (C). Irradiation with 100 Gy, 76 kV X-ray.

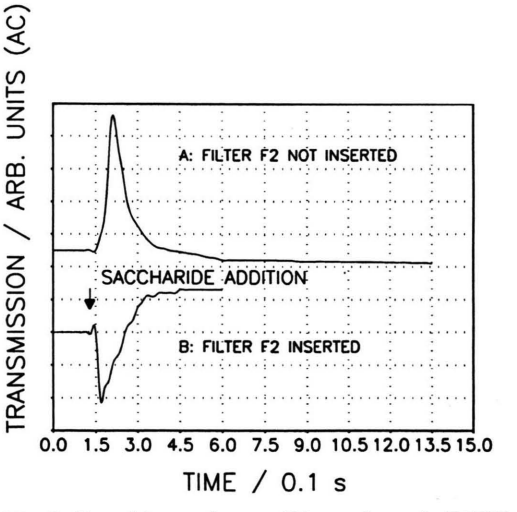


Fig. 3. Transition pulse at 420 nm through DPBF during solution of mannose, irradiated with 100 Gy, 76 kV X-ray. A: Superposed by the LL-pulse. B: LL-pulse avoided by filter.

furan (DPBF) reacts chemically with singlet oxygen [11]. Its rapid disappearance in solution can easily be monitored by a reduction of the DPBF absorption at 420 nm. For this measurement a transmission cell has been constructed (Figure 1). A tungsten band lamp (Typ Cool-White, Osram) was used as a light source. A band in the range 370–470 nm was selected with a filter combination F1 (GG 385/2 mm + BG 12/2 mm, Schott). DPBF is not soluble in water but in aceton, whereas saccharides are not soluble in aceton. A mixture of DPBF-aceton and water was used as a solvent. The increasing transmission caused by the decrease of DPBF concentration was observed with a photomultiplier (PM EMI 6256 B). The solvent was placed in a vessel. Saccharides were added by a dispenser. The transmission was registered as a pulse while irradiated sugar droped into the solvent.

Even DPBF emits a weak phosphorescence at 480 nm, when dissolved in aceton (Fig. 2, curve C). When mannose is dissolved in DPBF-aceton/H_2O, a strong lyoluminescence is observed (B). The integral lyoluminescence is about 10 times higher than the signal from dissolution in pure water (A). To avoid the registration of light of this reaction during the transmission measurement a second identical filter combination F2 was placed between the vessel and the PM tube.

The observed transmission pulse of the selected band (420 nm) is shown in Figure 3 (B). Three parts of the transmission curve can be distinguished. The first one is the DPBF transmission. An abrupt decrease of transmission is caused by scattering of light by the saccharide crystals. During the dissolution of the crys-

tals an increase is registered, resulting in a transmission which is sigificantly higher than before the LL-process, indicating a reduction of DPBF concentration in the solution as a result of the $^1O_2$-DPBF reaction. The transmission pulse superposed by the LL-pulse is shown in curve A for comparison.

By means of the chemical quenching of singlet oxygen in the reaction with DPBF the production of $^1O_2$ during the LL-process has been proved.

3.2. Influence of Reduced Singlet Oxygen Concentration on Lyoluminescence

Further investigations aimed at the decision whether $^1O_2$ is only a reaction product or even necessary for the LL-process itself.

β-carotene is an effective $^1O_2$ desactivator. The result is groundstate triplet oxygen ($^3O_2$) without O_2 consumption [18, 19]. Simultaneously the groundstate cis-β-carotene is transferred into the trans isomer:

$$\text{cis-}\beta\text{-carotene} + {}^1O_2 \rightarrow \text{trans-}\beta\text{-carotene} + {}^3O_2\,. \quad (3)$$

During this reaction no light emission occurs. This interaction was used to estimated the contribution of $^1O_2$ in the lyoluminescence process.

β-carotene is not soluble in water, but in aceton. As the direct mixing of β-carotene-aceton with water causes recrystalization of β-carotene, a solution of $7 \cdot 10^{-5}$ N β-carotene in 0.75 ml aceton and 0.75 ml water were injected simultaneously from separate injectors. Mannose was dissolved in a vessel (Fig. 4). The injection rate of the liquids was 0.8 ml/s each, and β-carotene was injected 0.1 s earlier. The injection of the solvent is better than the dissolving process, in which saccharides drop into the solvent, as an intense mixture of solid and liquid is achieved and even as the observation of the LL-pulse at its rise is less disturbed by emission light absorption in β-carotene-acetone (Figure 5). β-carotene is not stable against oxidation in $^3O_2$, especially in the presence of light. Therefore the measurements were performed with fresh β-carotene-aceton in the dark. Mannose was irradiated by 1–1000 Gy. A decrease of the LL by β-carotene was observed in the whole dose range. The PM-signal was reduced by about 80% relative to the lyoluminescence in pure water due to absorption in β-carotene and to quenching of $^1O_2$ (Figure 6).

The contributions of both effects were estimated. Mannose exhibits the LL-emission maximum at 480 nm. Here the main effect of β-carotene absorption

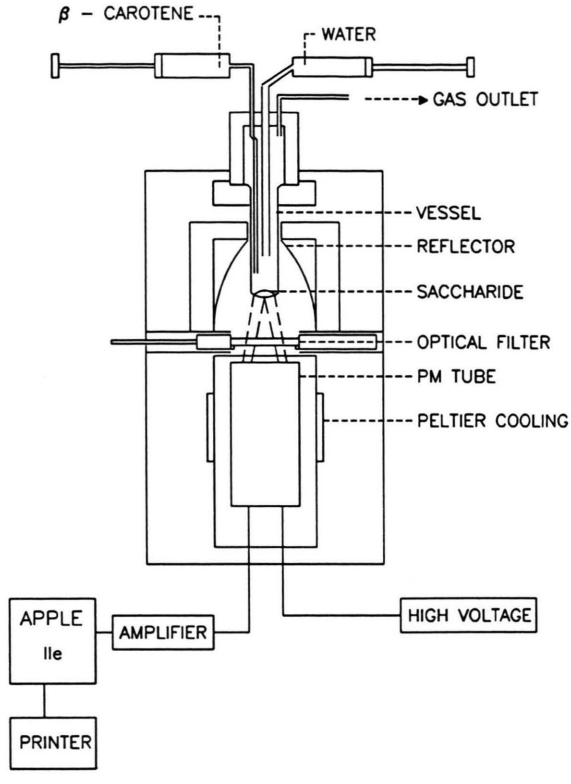


Fig. 4. Optical set-up for the registration of LL decrease by singlet oxygen quenching at β-carotene addition.

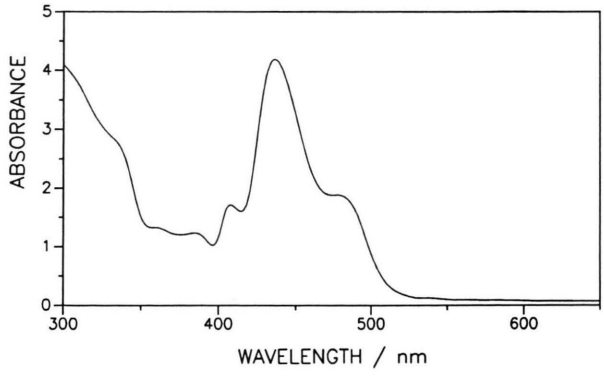


Fig. 5. Absorption spectrum of $7 \cdot 10^{-5}$ N β-carotene solution in aceton.

is expected with respect to the overlap of both emission and absorption, although the maximum of absorption is at about 430 nm. The molar extinction coefficient of the used $7 \cdot 10^{-5}$ N β-carotene at 480 nm was determined to $\varepsilon_{480} = 2 \cdot 10^4$ l/mol cm. It is 25% of the molar extinction coefficient published by Baugh et al.

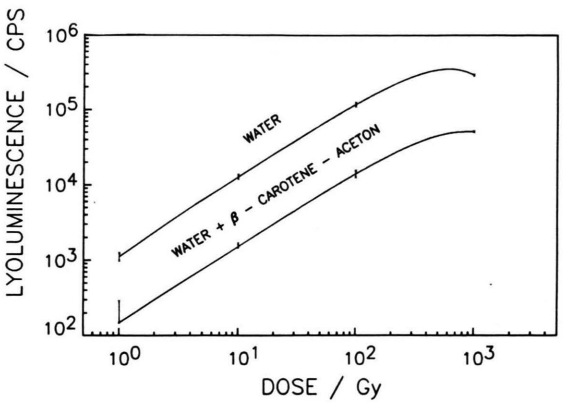


Fig. 6. Integral LL output of mannose as a function of X-ray dose (76 kV) at dissolution in pure water and in β-carotene/aceton + water.

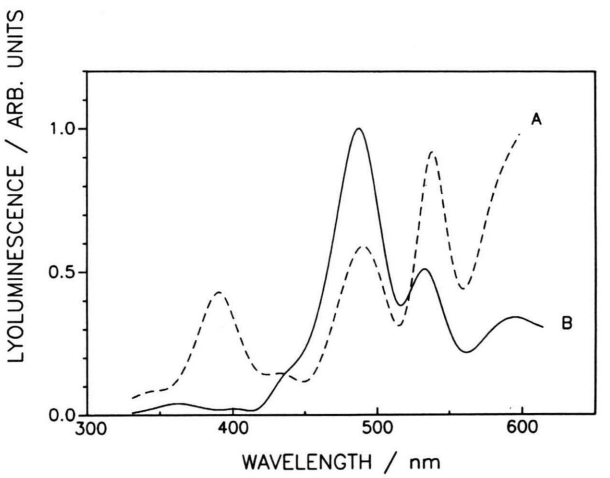


Fig. 7. LL-emission spectra of fructose (A) and mannose (B) after irradiation with 100 Gy X-ray, 76 kV. Solvent: Water, pH 6.97.

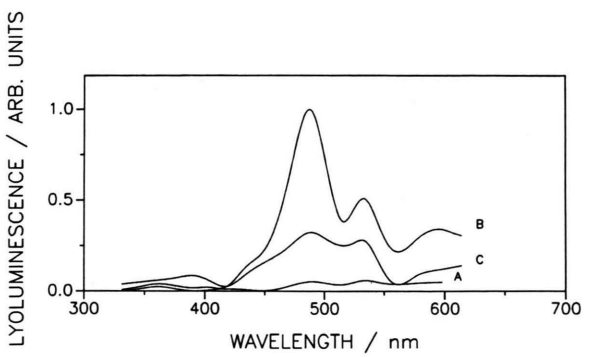


Fig. 8. LL-emission spectra of mannose at different oxygen concentrations in water (pH 7.1). 100 Gy X-ray, 76 kV. A: 33.5 mg O_2/l, B: 8.3 mg O_2/l, C: 2.6 mg O_2/l.

in an undefined solution [6]. Assuming that the reduction is generated only by light absorption, a conservative estimation of the resulting light output at the PM was performed. The calculated absorption in the solvent is about 1 order of magnitude lower than that measured by Baugh, who reported 80% absorption. Therefore quenching of a light emission generating species, especially $^1O_2$, must be the main reason for LL reduction. Assuming a homogeneous distribution of the solid in the liquid during solvent injection and an enhancement by aceton of 10%, both contributions to the reduction were estimated to 78.5% quenching of $^1O_2$ and 10.5% absorption of LL-emission by β-carotene, respectively.

By this estimation the light emission in the LL of saccharides via $^1O_2$ as proposed by Ettinger and Puite [4] as one of several possible mechanisms has been proved.

3.3. Investigation of LL-Spectra of Saccharides

The LL emission spectra of mannose and fructose were registered in a separate cage [1] at higher resolution (Fig. 7), using a set of edge filters ($\Delta\lambda = 30$ nm). The LB 950 read-out technique has been applied.

The spectra of both mannose and fructose exhibit peaks at 385 nm, 490 nm, 535 nm, where Ettinger and Puite [4] observed only a broad band. These emission peaks may be attributed, in accordance to Khan and Kasha [16], to the following transitions from collisional pairs of singlet oxygen ($^1O_2^* - {}^1O_2^*$):

$$^1\Delta_g\,{}^1\Sigma_g \rightarrow {}^3\Sigma_g\,{}^3\Sigma_g \qquad 490\text{ nm},$$
$$^1\Delta_g\,{}^1\Delta_g \rightarrow {}^3\Sigma_g\,{}^3\Sigma_g \qquad 520\text{ nm},$$
$$^1\Sigma_g\,{}^1\Sigma_g \rightarrow {}^3\Sigma_g\,{}^3\Sigma_g \qquad 360\text{ nm}.$$

In order to check whether this collisional oxygen emission process is accompanied even by the concurring carbonyl emission, the emission spectrum of mannose was measured at low oxygen concentration as well (Figure 8). Here the LL-maxima of the collisional pair emission are reduced by one of magnitude as a function of oxygen concentration. In this case even the desactivation of excited carbonyl bindings should be reduced, but no additional carbonyl emission at 430–490 nm was found. Therefore a superposition of the emission of both carbonyl and the $(^1O_2^*)_2$ ar normal oxygen concentration (8.3 mg/l) may be excluded.

4. Conclusions

Lyoluminescence emission is a consequence of a radical reaction. Ettinger et al. have proposed a model for the LL of saccharides in Russel's scheme in which peroxyl radicals may be created during solution with succeeding disproportionation and production of singlet oxygen. Collisional pairs of singlet oxygen were assumed to be the light emitters. In this investigation the presence of singlet oxygen in the LL of saccharides and its necessity for the light emission has been proved. The peaks of the measured LL-spectra are in accordance with the light emission by pairs of singlet oxygen. Therefore collisional pairs of singlet oxygen can be identified as the LL-emission centres in saccharides. As a consequence for dosimetric application, the oxygen content of the solvent must be carefully controlled. Herewith the statistic error has been reduced from 20% to 10%, resulting in an improvement of the lower detection limit by a factor 2.

Acknowledgements

This research has been supported by the German Federal Ministers of Defence and of the Interior. The authors are grateful to Dipl.-Phys. Thomas Nickel for the discussions. This work formed part of thesis (Gießen, D 26) for T.S.

[1] Th. Nickel, E. Pitt, A. Scharmann, and T. Suprihadi, Radiat. Prot. Dosim. **33,** 233 (1990).
[2] G. Ahnström and G. Ehrenstein, Acta Chem. Scand. **13,** 855 (1959).
[3] R. F. Vassil'ev, Prog. React. Kin. **4,** 305 (1967).
[4] K. V. Ettinger and K. J. Puite, Int. J. Appl. Radiat. Isot. **33,** 1115 (1982).
[5] G. A. Löfroth and Ch. Kim, Radiat. Eff. **3,** 217 (1970).
[6] P. J. Baugh and P. Laflin, Radiat. Phys. Chem. **16,** 51 (1980).
[7] G. Russel, J. Amer. Chem. Soc. **79,** 3871 (1957).
[8] K. V. Ettinger, A. R. Forrester, and J. R. Mallard, Lyoluminescence Dosimetry. Physicochemical Basis and Applications. Proc. Internat. Sym. Bio-Medical Dosimetry, Vienna 1980 (IAEA-M-249/15), p. 533.
[9] K. V. Ettinger, R. W. Rowe, J. R. Mallard, A. Takavar, J. P. Sephton, and D. I. Thwaites, The Lyoluminescence of Amino Acids, Proteins, Nucleic Acids and Its Applications. Proc. 5th Internat. Conf. on Luminescence Dosimetry, Sao Paulo 1977 (A. Scharmann, ed.), p. 162.
[10] R. E. Kellog, J. Amer. Chem. Soc. **91,** 5433 (1969).
[11] D. R. Kearns, Solvent and Solvent Isotope Effects on the Lifetime of Singlet Oxygen, in: Singlet Oxygen (H. H. Wasserman, ed.), Academic Press, New York 1979, p. 115.
[12] J. W. Ellis and H. O. Kneser, Z. Phys. **86,** 583 (1933).
[13] H. Salow and W. Steiner, Z. Phys. **99,** 137 1936).
[14] V. I. Dianov-Klokov, Opt. Spectrosc. **16,** 224 (1964).
[15] V. G. Krishna, J. Chem. Phys. **50,** 792 (1969); **51,** 2140 (1969).
[16] A. U. Khan and M. Kasha, J. Amer. Chem. Soc. **92,** 3293 (1970).
[17] M. Kakano, K. Takayama, Y. Shimizu, Y. Tsuji, Y. Inaba, and T. Migita, J. Amer. Chem. Soc. **98,** 174 (1976).
[18] Ch. S. Foote and R. W. Denny, J. Amer. Chem. Soc. **90,** 6233 (1968).
[19] Ch. S. Foote, Quenching of Singlet Oxygen, in: Singlet Oxygen (H. H. Wasserman, ed.), Academic Press, New York 1979, p. 137.