ELSEVIER

Photo- and radiochemiluminescence: reductive chemiluminescence of lucigenin by photo- or radiooxygenated amines and amides

K. Papadopoulos[a], T. Triantis[b], D. Dimotikali[b], J. Nikokavouras[a,*]

[a]*National Research Centre for Physical Sciences "Demokritos", Institute of Physical Chemistry, 15310 Ag. Paraskevi, Athens, Greece*
[b]*National Technical University, Chemical Engineering Department, Iroon Politechniou 9, 15780 Zografou, Athens, Greece*

Abstract

Addition of photo- or radiooxygenated amines or *N,N*-dialkylamides to aqueous lucigenin results in efficient chemiluminescence. Chemiluminescence quantum yields for amines are two orders of magnitude higher than those of dialkylamides for the same illumination or irradiation time and can become as high as 4×10^{-3} einstein/mol. The mechanisms of the photo- or radiooxygenation and chemiluminescence are discussed. The combined photolysis or radiolysis-chemiluminescence reactions constitute light storage systems and prospective radiation dosemeters. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Chemiluminescence; Lucigenin; Photolysis; Radiolysis

1. Introduction

Few light storage systems are known in the sense that light is absorbed and light is released or in other words systems in which photolysis [1–4] or radiolysis [5–8] is followed by chemiluminescence (CL). In two of the photolyzed systems [4,5], CL can be triggered at will while in the rest CL follows the light absorption spontaneously. The first of the controlled systems is based on the formation of dimers [4] while the second is based on coupling products with the solvent-reagent and is several thousand times stronger [5]. Similarly, we have controlled radiochemiluminescent systems which are quite efficient [5,8].

It is known that lucigenin **1** can be reduced electrochemically [9,10] or via electron transfer reactions from nucleophiles [11] to *N,N*-dimethylbiacridylidene (DBA) **2** and produce light (Scheme 1). It is realized and this will become evident in the following sections that this is a very complex system with several reactions proceeding simultaneously, yet it was chosen at the expense of the more easily understood acridines because of the more challenging reductive CL involved.

In the present work we wish to report reductive CL reactions of lucigenin with photo- or radiooxygenated alkylamines (*n*-butylamine, diisopropylamine, triethylamine) and *N,N*-dialkylamides such as *N,N*-dimethylformamide

(DMF) and *N,N*-dimethylacetamide (DMA) and discuss probable photolysis/radiolysis and chemiluminescence mechanisms.

## 2. Experimental techniques

2.1. Reagents

Lucigenin **1** and *N*-methylacridone (NMA) **3** were purchased from Aldrich and used without further purification. *N,N*-dimethylbiacridylidene (DBA) **2** was synthesized in our laboratory according to a known procedure [12]. Amines and dialkylamides were dried and purified by the standard procedure [13]. Working solutions were freshly made, and were not employed for periods over one day (lucigenin solutions in amide solvents decompose slowly after few days).

2.2. Equipment

Absorption spectra were run on a JASCO V-560 spectrophotometer. Fluorescence spectra were recorded on a JASCO FP-777 Spectrofluorimeter (Scan speed 200 nm min^{-1}, emission band 5 nm); CL spectra were run on the JASCO FP-777 spectrofluorimeter with the excitation source off, employing wide slits (20 nm) and a scanning rate of 1000 nm min^{-1} (2.0 ml aqueous lucigenin solution,

*Corresponding author. Fax: +30-1-6511766.
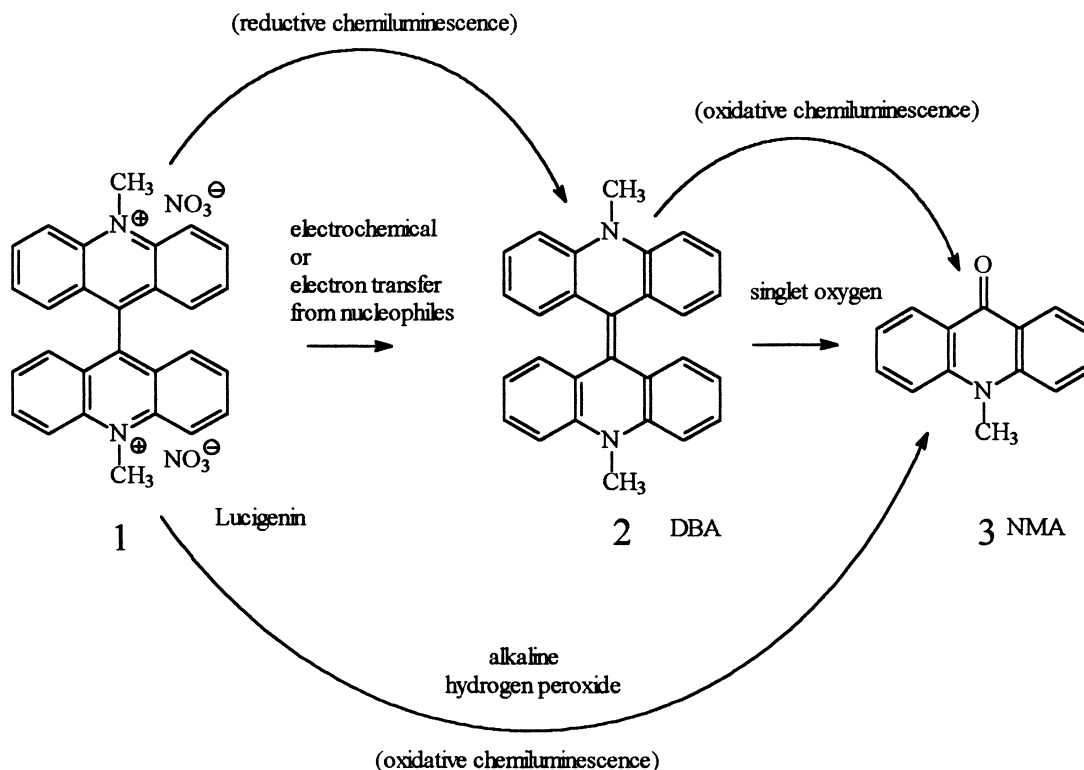
Scheme 1. Chemiluminescence reactions of lucigenin **1** to *N,N*-dimethylbiacridylidene **2** (reductive CL) and *N*-methylacridone **3** (oxidative CL).

10^{-4} M and 1.0 ml photo- or radiooxygenated amine or amide). GC (Carlo Erba, GC 8060) and Mass spectra (Micromass, Platform II, quadrupole) were determined on a Lab instrument. Finally, the electron spin resonance (esr) spectra were measured with a Brucker spectrometer at $-196°C$ at 9.32 GHz.

2.2.1. Photo- or radiooxygenation

A square quartz cuvette, with oxygen saturated alkyl-amines or amides (5 ml) was irradiated for $4\frac{1}{2}$ h at 25°C with the full spectrum of a Xenon-lamp. (Oriel, 1000 Watt). The photochemical reaction was monitored using a Jasco UV 560 spectrophotometer (λ_{max}281 nm).

γ-Radiolysis experiments were performed at room temperature in a ^{60}Co, 6500 Ci, Gamma Chamber (4000 A, Isotope Group, Bhaba Atomic Research Centre, Trombay, India). 25 ml oxygen saturated samples of liquid alkyl-amines or *N,N*-dialkylamides were irradiated for one to seven days. Similar products were obtained from both photolysis and radiolysis, mainly amide dimers such as *N,N*-dimethylformamide dimer (M$^+$, 144) as well as mono- and dialkyl derivatives of amines and amides (dimethyl-amine M$^+$ 45, *N*-methylformamide M$^+$ 59) The dose rate was determined with Fricke's dosemeter [14] and was found equal to 30.03 Gy min^{-1}.

2.2.2. Chemiluminescence measurements

These were performed on a LKB 1250 Bio-Orbit lumin-ometer with the timer circuitry disconnected. The cell's

jacket was thermostatically controlled with the aid of a constant temperature bath-circulator and the temperature was maintained at $25.0\pm0.1°C$. The light reactions were started by adding 250 µl photo- or radiooxygenated amine into an aqueous solution of lucigenin (250 µl, 10^{-5} M, $\Phi_{CL}\approx10^{-3}$ einstein/mol). As amides were less efficient ($\Phi_{CL}\approx10^{-5}$ einstein/mol) both the volumes and concentra-tions had to be increased; in this case 500 µl of aqueous lucigenin 10^{-4} M and 500 µl of photo- or radiooxygenated amide samples were employed. For comparison purposes, it should be noted that non-photolyzed or radiolyzed amines gave rise to CL quantum yields in the order of 10^{-6} to 10^{-7} einstein/mol for amides, while the CL signals were similarly poor (30 times weaker for amides and 500 times for amines. It should also be noted that when lucigenin is dissolved in DMF rather than water, the CL quantum yield drops from over 10^{-3} to 10^{-7} einstein/mol. Said quantum yields were calculated from the light intensity-time integrals on comparison with the Luminol Standard [15] which served as an absolute photon source under the same geometry.

2.2.3. Chemiluminescence products

The photo- or radiooxygenated solutions of amines or amides were added to aqueous solutions of lucigenin and the resulting mixtures were left in the dark for 5 h. Product identification was performed by comparison of the UV- and fluorescence (emission and excitation) spectra with those of an authentic NMA **3** or DBA **2** sample, indicating that DBA is the major CL product.

3. Results and discussion

3.1. Photolysis/radiolysis

Because of the similarity of the radiolysis and photolysis mechanisms they will now be discussed together. A differentiation is, in this case however expected between radiolysis and photolysis. The initial energy deposition processes and hence the primary radical distribution are different for high energy ionizing radiation and light. In the former case, hydrogen radicals ($H^{\cdot}$), aminyl radicals ($R_2N^{\cdot}$) and solvated electrons are directly produced which then react with oxygen to give unstable products [16–23] such as nitroxyl radicals and their disproportionation products, hydroxylamines and nitrones. Formation of superoxide/perhydroxyl ($O_2^{-\cdot}/HO_2^{\cdot}$) or ($OH^{\cdot}$) radicals [24], produced on radiolysis and decomposing further to stable oxygen derivatives such as hydrogen peroxide and oxygen [25–27] should also be considered (Scheme 2). In the case of photolysis it is reasonable to assume that dialkylamides or alkylamines in the excited state build first an adduct with oxygen which after electron and proton transfer from amine/amide to oxygen gives radical pairs which can be stabilized by fragmentation, disproportionation, dimerization or coupling reactions (Scheme 2). Indeed, GC–MS techniques revealed the presence of products with molecular weights corresponding to DMF dimers (M^+ 144) and dimethylamine (M^+ 45). The need for water in the photo- or radiochemiluminescnce reactions is shown by the transient broad band absorption appearing for ca. 20 s at 400–900 nm on addition of water to the photo- or radiolyzed amides or amines at room temperature (Fig. 1). In this time period epr reveals the presence of a peak with g 2.009, characteristic of simple radicals such as those ($HO_2^{\cdot}$ or $OH^{\cdot}$) of Scheme 2. The light or radioactivity energy storage step is therefore, formation of hydroxylamines and nitrones (UV spectra of these compounds have been reported between 250 and 310 nm [28] and also appear in our reaction mixtures on photo- or radiolysis) from amines (photo- or radiolysis of amides also leads to amines) and the mixture of said photoproducts in amines or amides is stable for at least several weeks. On addition of this mixture to aqueous lucigenin, reaction with water will form the peroxide-amine ion pair-reagent (Scheme 2) for the lucigenin CL, but this is a matter of the section that follows.

3.2. Chemiluminescence

The chemiluminescence of lucigenin **1** was first reported in 1935 by Gleu and Petsch [29]. They observed intense chemiluminescence when lucigenin was treated with hydrogen peroxide in basic solutions. Eventually NMA **3** was conclusively identified as the primary emitter (oxidative CL) (Scheme 1). By electrogenerated chemiluminescence of lucigenin, Legg and Hercules [9,10] have shown that in addition to NMA a long wavelength component is also observed and is assigned to either lucigenin or DBA **2** (oxidative and reductive CL). In the reaction of lucigenin with several nucleophiles in the absence of molecular oxygen or oxidizing agents reductive CL has exclusively been obtained by two electron transfer from nucleophiles to lucigenin, but the CL quantum yields were much lower [11] compared to our results. In our current experiments, the addition of photo- or radiooxygenated *N,N*-dimethylamides or alkylamines such *n*-butylamine, diisopropylamine and triethylamine to aqueous solutions of lucigenin results in intense reductive CL in the order of 10^{-2} einstein/mol with DBA as the emitting species. Addition of the above amides/amines to solutions of lucigenin in aprotic organic solvents such as DMF or DMSO results only in weak CL (Φ_{CL} ca. 10^{-6} einstein/mol), so water is necessary for CL in this system, even if only for the formation of the peroxide-amine
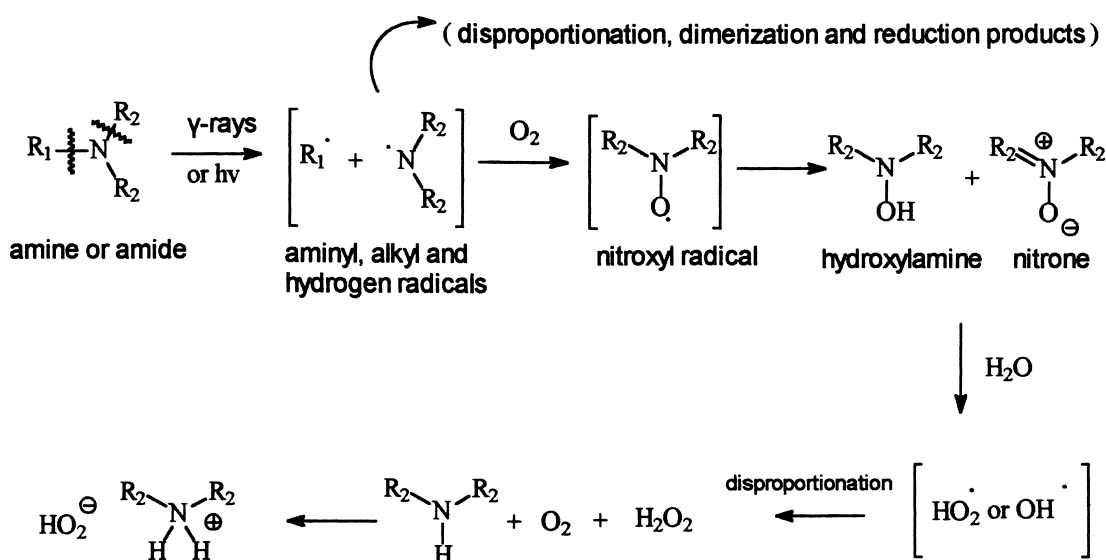
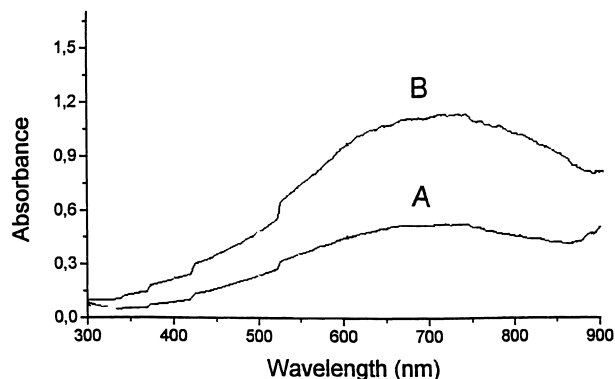Scheme 2. Proposed mechanism of the photo- and radiooxidation of amines/amides.

Fig. 1. Absorption spectra on addition to water-DMF of diisopropylamine photooxygenated for 4 h (A) and radiooxygenated for 18 h (B). These are transient peaks lasting no more than 20 s.

ion pair of Scheme 2. Immediately after addition of the photo- or radiolyzed amides/amines to the aqueous lucigenin solutions the transient species described in the preceding section is produced showing a broad absorption band at a longer wavelength (Fig. 1), and an esr spectrum with g value of 2.009 not observed in organic solutions of lucigenin (Fig. 2). We attribute this band to the perhydroxyl or hydroxyl radical [30] argued earlier which can lead to charge transfer complexes of the perhydroxyl or hydroxide anion (produced via disproportionation) with lucigenin **4** (Scheme 3). The formation of similar CT complexes of lucigenin has also been observed in reactions with other anions/nucleophiles [11]. We repeated said experiments with the amines of the present work without photo- or radiolysis and the CL quantum yields were lower by ca. 2 orders of magnitude.

The optimum lucigenin concentration for maximum CL was 10^{-5} M and it is interesting to note that CL is far more efficient in dialkylamines (diisopropylamine, Φ_{CL}, 4.4×10^{-3} einstein/mol) than in dialkylamides (DMF, Φ_{CL} 1.1×10^{-4}, DMA, Φ_{CL} 2.4×10^{-5} einstein/mol), irradiation time of 18 h. The duration of light emission as reflected on the CL intensity-time diagrams (not shown) depends on
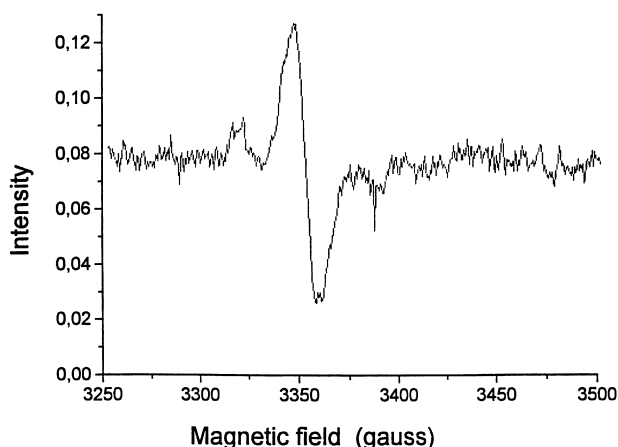
the solvent and photo- or radiolysis time and ranges from ca. 10 min (amines) up to 3 h (amides). This duration refers to the high rate transformation of lucigenin to DBA and not to the slow self luminescence of DBA leading to NMA.

As mentioned earlier, this is indeed a very complex CL system. To begin with, addition of the photo/radiooxygenation products of the present work to aqueous solutions of lucigenin gives rise to emission of light whose spectral distribution is that of the DBA fluorescence (505 nm). The excitation and fluorescence spectra of the spent reaction mixture are also those of DBA with a very small contribution by NMA (413, 436 nm), product of the slow "self luminescence" of DBA. In addition to these two "light sources", reaction of lucigenin with DBA is also CL although neither quantum yields nor spectra have been given [31]. Here, a new emission appears at a longer wavelength (550 nm) which after 30 min starts to fade and the NMA emission becomes evident (Fig. 3). Amines and hydrazines have been suggested to trigger lucigenin CL [11] while primary and secondary amines in protic solvents have been shown to reduce lucigenin to DBA [32,33]. In the present experiments photo- or radiooxygenated amines (diisopropylamine, n-butylamine and triethylamine, pure solvent, produce reductive CL leading to DBA with quantum yields higher by 2 orders of magnitude. This is true for the low concentrations of hydrogen peroxide in these amines dictated by the mechanism of Scheme 2, as externally added higher concentrations (C $\geq$ 1 M) give rise to reductive and oxidative CL leading to a mixture of DBA and NMA. In conclusion, the CL of lucigenin on reaction with photolyzed/radiolyzed amides/amines is probably due to the amines added or those produced on photo- or radiolysis of the amides (e.g. DMF→dimethylamine) together with the hydrogen perox-

Fig. 2. The electron spin resonance spectrum of the free radical obtained from radiooxygenated diisopropylamine in aqueous solution at −196°C, at 9.39 GHz.

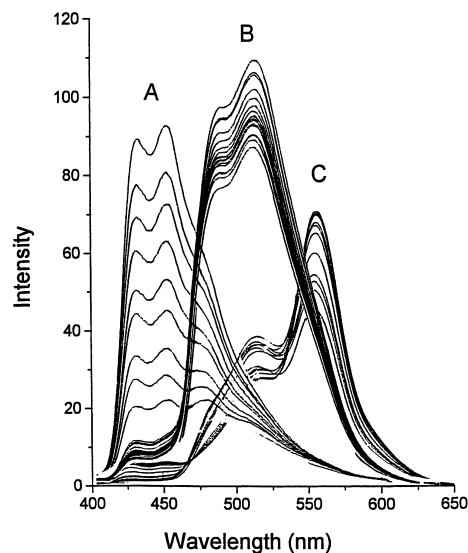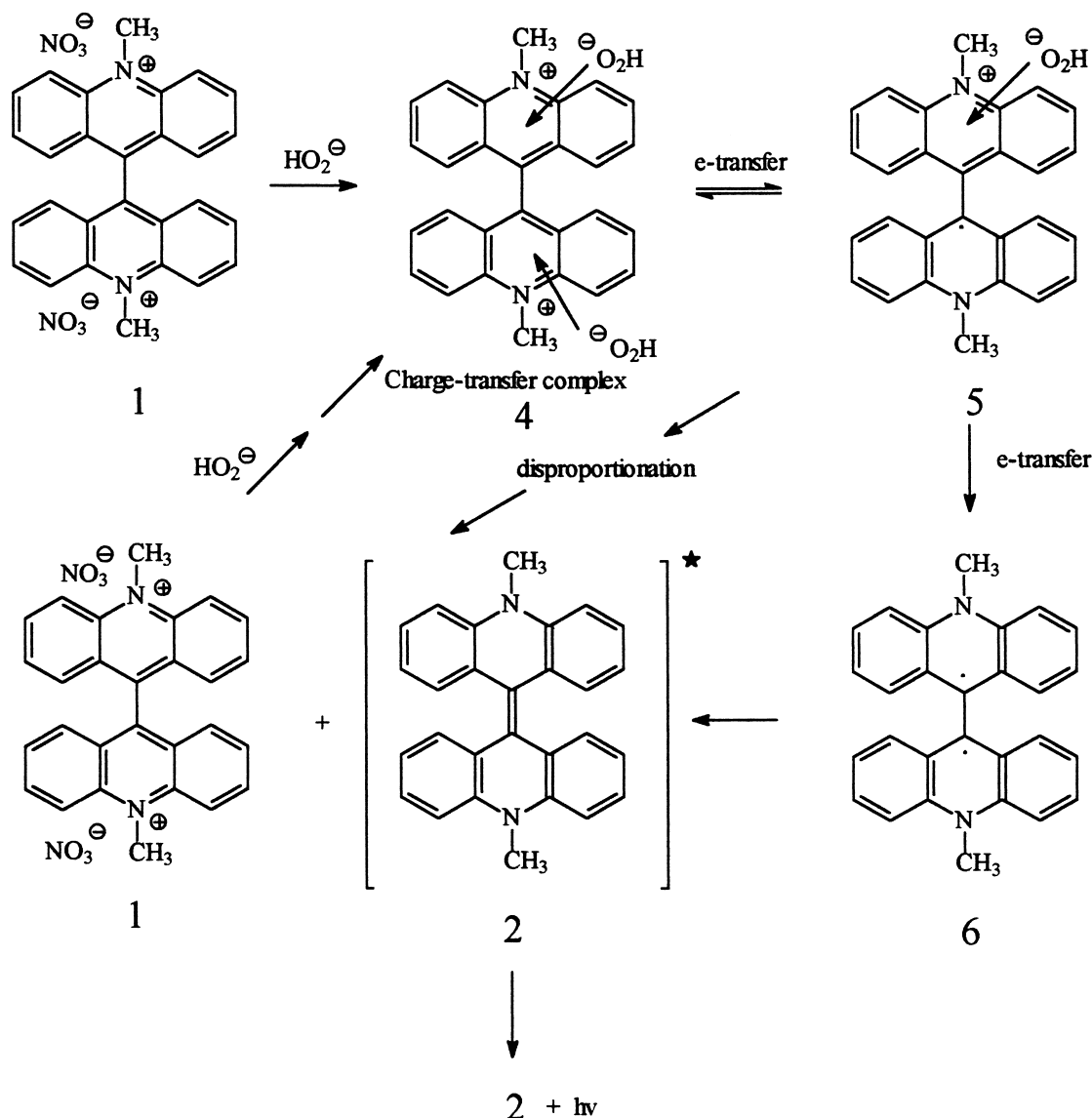Fig. 3. Successive fluorescence spectra of the CL reaction mixtures of lucigenin with (A) dimethylamine/hydrogen peroxide (C$\geq$1 M), (B) with photo-or radiooxygenated amides and (C) with N,N-dimethybiacrydylidene in DMF/water.

Scheme 3. Proposed reductive chemiluminescence reaction mechanism of lucigenin with photo- or radiooxygenated amides/amines.

ide anion formed on hydrolysis of the products of the photo- or radiolyzed amines/amides (Scheme 2) and should be noted here that addition of the amines of the present work together with small concentrations of hydrogen peroxide (lower than 1 M) to aqueous lucigenin also results in reductive CL, with comparable efficiencies. The possible contribution of the hydroxyl anion formed in small quantities during photo/radiooxygenation reactions should be also taken into account. All three species are electron donors and can give rise to reductive CL of lucigenin.

3.3. Prospects for novel radiation dosemeters

Addition of the radiooxygenated amides to aqueous lucigenin solutions results in CL whose intensity-time integrals are, as expected, a function of radiation absorbed. The light integrals thus obtained are directly proportional to the dose
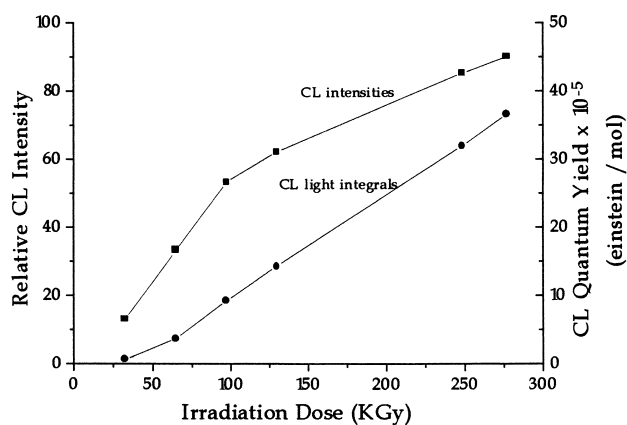

Fig. 4. CL light intensities/integrals of lucigenin ($C=10^{-4}$ M) with radiooxygenated DMF plotted versus absorbed γ-ray dose.

absorbed and this is shown in Fig. 4, where the dose absorbed was determined with the aid of Fricke's actinometer [10] as described earlier. Under our experimental conditions this plot shows that this reaction can serve as a γ-radiation dosemeter and most probably also for other ionizing radiations capable of radiolyzing the present solvents (internal dosemeters), in the region of 30–280 KGy. Lower scales and higher sensitivities were obtained when amines were employed instead of amides.

4. Conclusions

In conclusion, photolysed or γ-radiolysed liquid amines or amides react with aqueous lucigenin giving rise to chemiluminescence with quantum yields comparable to the classical CL systems as reaction with photo/radiooxygenated diisopropylamine results in a CL quantum yield of ca. 10^{-2} einstein/mol. The novel CL reactions could be of use in speciallized analytical applications. Also it was hopefully shown that said reactions can be employed as radiation dosemeters without isolation of the radioproducts. Finally, said reactions constitute light storage systems, in which formation of the photo- or radioproducts of the present work – stable for several weeks – constitutes the light storage step and the CL on addition to aqueous lucigenin, the light release step.

Acknowledgements

References

[1] T. Hayashi, K. Maeda, Bull. Chem. Soc. Jpn. 36 (1963) 1052.
[2] K. Maeda, H. Ozima, T. Hayashi, Bull. Chem. Soc. Jpn. 38 (1965) 76.
[3] S. Boyatzis, J. Nikokavouras, J. Photochem., Photobiol. A 44 (1988) 335.
[4] J. Stauff, G. Stark, Z. Naturforsch. 41b (1986) 113.
[5] K. Papadopoulos, D. Dimotikali, J. Nikokavouras, J. Photochem. Photobiol. A 103 (1997) 51.
[6] J. Nikokavouras, A.E. Mantaka, D.G. Marketos, N.T.H. Rakitzis, G. Vassilopoulos, Z. Physik. Chem. N. F. 78 (1972) 76.
[7] A.E. Mantaka, D.G. Marketos, N.T.H. Rakitzis, G. Vassilopoulos, J. Nikokavouras, Z. Physik. Chem. N.F. 85 (1973) 106.
[8] K. Papadopoulos, J. Lignos, M. Stamatakis, D. Dimotikali, J. Nikokavouras, J. Photochem. Photobiol. A 115 (1998) 137.
[9] K.D. Legg, D.M. Hecules, J. Am. Chem. Soc. 91 (1969) 1902.
[10] K.D. Legg, D.W. Shive, D.M. Hercules, Anal. Chem. 44 (1972) 1650.
[11] K. Maeda, T. Kashiwabara, M. Tokuyama, Bull. Chem. Soc. Jpn. 50 (1977) 473.
[12] K. Papadopoulos, J. Nikokavouras, J. Prakt. Chem. 335 (1993) 633.
[13] D.D. Perin, W.L.F. Armarego (Eds.), 3rd ed., Pergamon Press, Oxford, 1989, p. 157.
[14] J.W.T. Spinks, R.J. Woods (Eds.), 3rd ed., John Wiley, New York, 1990, p. 71.
[15] J. Lee, A.S. Wesley, J.F. Ferguson, H.H. Seliger, in: F.H. Johnson, Y. Haneda (Eds.), Bioluminescence in Progress, Princeton University Press, NJ, 1966, p. 35.
[16] J.Q. Adams, S.W. Nicsic, J.R. Thomas, J. Chem. Phys. 45 (1966) 654.
[17] A. Hudson, H.A. Hussain, J. Chem. Soc. B (1967) 1299.
[18] J.T. Weil, J.J. Windle, Nature 217 (1968) 842.
[19] R. Poupko, A. Loewenstein, B.L. Silver, J. Am. Chem. Soc. 93 (1971) 580.
[20] H.G. Aurich, J. Can. Chem. 60 (1982) 1414.
[21] M.M. Mosoba, I. Rosenthal, P. Riesz, J. Can. Chem. 60 (1982) 1493.
[22] R-M. Dupeyre, A. Rassat, J. Am. Chem. Soc. 88 (1966) 3180.
[23] D.F. Bowman, T. Gillan, K.U. Ingold, J. Am. Chem. Soc. 93 (1971) 6555.
[24] D.T. Sawyer, J.S. Valentine, Acc. Chem. Res. 14 (1981) 393.
[25] B.H.J. Bielski, D.E. Cabell, R.L. Arudi, J. Phys. Chem. Ref. Data 14 (1985) 1041.
[26] B.H.J. Bielski, R.L. Arudi, Anal. Biochem. 133 (1983) 170.
[27] G.W. Eastland, M.C.R. Symons, J. Phys. Chem. 81 (1977) 1502.
[28] J. Hamer, A. Macaluso, Chem. Rev. 64 (1964) 473.
[29] K. Gleu, W. Petsch, Angew. Chem. 48 (1935) 57.
[30] B.H.J. Bielski, J. Am. Chem. Soc. 83 (1961) 4467.
[31] R. Maskiewicz, D. Sogah, T.C. Bruice, J. Am. Chem. Soc. 101 (1979) 5355.
[32] K. Papadopoulos, J. Nikokavouras, Tetrehedron Lett., (1993) 1371.
[33] K. Papadopoulos, J. Nikokavouras, J. Prakt. Chem. 336 (1994) 506.