Studies on the photostoragechemiluminescence of aromatic ketones with reactive oxygen species Prospects for analytical applications

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Abstract

It is believed that chemiluminescence (CL) of photolyzed aromatic compounds proceeds in alkaline media and in the presence of oxygen, with various reactive oxygen species (ROS). In order to prove this hypothesis we have investigated the contribution of chemically produced ROS, such as hydroxyl radicals, singlet oxygen as well as superoxide radical anions on CL reactions of photolyzed aromatic compounds. The results of this investigation show, indeed, that the light emission (CL) is due to the reaction of photoproducts of aromatic ketones with singlet oxygen or superoxide radical anion. The contribution of hydroxyl radicals in this CL reaction is insignificant. Although, the CL light efficiency of the above reactions is not high, the results of this study can be used for the quantitative determination of some of these ROS as well as for the aromatic compounds down to few parts per billion.
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1. Introduction

Photostoragechemiluminescence (PSCL) is used to describe a process, which involves storage of a part of the light energy in a chemical system; the stored energy can be later released by suitable chemical triggering. Such combinations of photochemical reactions with chemiluminescence (CL) can be considered as light storage systems in the sense that 'light' is absorbed and light is re-emitted. We have recently reported that exposure of azaaromatics in *N*,*N*-dialkylated amides even to the diffuse light of the laboratory or γ-irradiation results in photolysis or γ-radiolysis, while subsequent addition of strong bases to said spent mixtures gives rise to very efficient CL [1–4]. In the present work, we have extended our results further to aromatic ketones, which after photolysis give CL with alkali hydroxides in the presence of oxygen. Such emission has been earlier observed and has been attributed to the decomposition of the inter- and/or intra-molecular peroxides produced during the photolysis step [5] or from the reaction of the photolyzed products with reactive oxygen species (ROS) produced from oxygen in alkaline solutions. The first case is true only for oxygenated aromatic compounds, while for deaerated solutions, as in our work, we believe that the second hypothesis is true. Indeed, the reaction of photolyzed aromatic compounds (dimerization, disproportionation and coupling) with chemically produced ROS, such as: (a) *hydroxyl radicals* (ferrous sulphate and hydrogen peroxide, Fenton-reaction), (b) sodium hypochlorite and hydrogen peroxide (*singlet oxygen*) and (c) potassium superoxide (superoxide *radical anion*) results in emission of light. At this point, it should be noted that in aprotic polar solvents stable solutions of superoxide anion can be prepared (a) by the base-induced decomposition of hydrogen peroxide [6,7] or by the addition of hydroxides to oxygen saturated amine solutions [8]. Within the frame of this work we used only aromatic compounds without reactive hydrogen atoms at the α-position to the carbonyl group, to avoid more photo- or CL products that would have complicated the mechanism [9–11]. As aromatic ketones, especially, polycondensed aromatic ones have fluorescence quantum yields higher than most other carbonyl compounds, particularly aliphatic ones, we anticipated that free-radical reactions generating even

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small yields of excited aromatic ketones could be studied without the conventional addition of fluorescent sensitizers, and simplify the analytical applications pursued.

2. Experimental techniques

2.1. Reagents/equipment

All aromatic ketones, xanthone, fluorenone, anthrone, acridone, *N*-methylacridone, dibenzosuberenone and the oxidizing reagents potassium superoxide, sodium peroxide and sodium hypochlorite (10–13%) were purchased from Aldrich and used without further purification. Ferrous sulphate hexahydrate (extra pure) and hydrogen peroxide (for analysis, 30%, w/v) used for the production of hydroxyl radicals, were purchased from Merck (Germany) and Panreac (Spain), respectively. Sodium hydroxide was used as 2.0 M aqueous solution. Working solutions were freshly made. Absorption spectra were run on a JASCO V-560 spectrophotometer. Fluorescence spectra were recorded on a JASCO FP-777 Spectrofluorimeter. GC/MS spectra were performed on an Agilent 5973N mass spectrometer. Samples were injected pulsed splitlessly into an Agilent 6890 gas chromatograph equipped with a J&W HP-5MS 5%, phenyl-methylsiloxane, 30 m × 0.25 mm × 0.25 μm film thickness capillary column. Flow rate of helium was 1 ml/min. The oven was programmed as follows: isothermal at 50 °C for 4 min, from 50 to 150 °C with 5 °C/min and held for 10 min, then from 150 to 220 °C with 10 °C/min and isothermal at 270 °C for 7 min with 10 °C/min. The electron energy was set at 70 eV, the ion source temperature was maintained at 230 °C and the quadrupole temperature at 150 °C.

2.2. Photolysis

A square quartz cuvette, with deaerated aromatic ketones in DMF (5 ml, 10^{-4} M) were irradiated at 25 °C with the full spectrum of a xenon lamp (Oriel, 1000 W) in an Oriel Instrument photolysis apparatus until the characteristic UV bands of ketones (anthrone: 390, 370 and 353 nm; *N*-methylacridone: 405 and 385 nm; acridone: 398 and 377 nm; fluorenone: 325, 313 and 298 nm; xanthone: 341 and 329 nm; dibenzosuberenone: 357 and 311 nm) disappeared; photolysis time, 40–60 s. It should be mentioned that the use of a cut-off filter at 305 nm in the above experiments did not affect significantly the CL efficiency.

2.3. CL measurements

These were performed on a 1250 Bio-Orbit Luminometer with the timer circuitry disconnected. For CL measurements, optimization experiments were performed for all oxidizing agents and found, to be 2 M for sodium hydroxide (Fig. 1) and 0.01 M for hydrogen peroxide, ferrous sulphate and sodium hypochlorite for hydroxyl radicals and singlet oxygen production, respectively. The light reactions
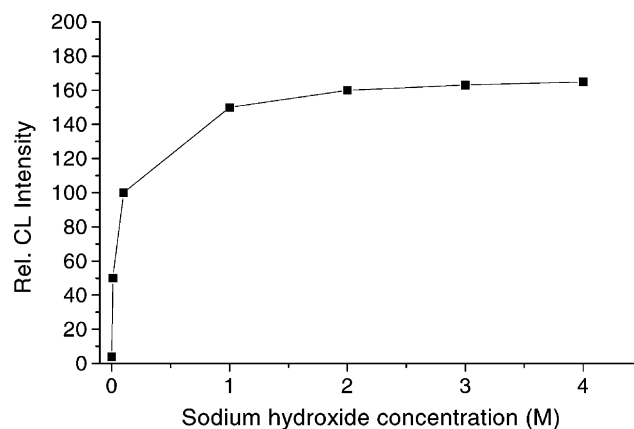

Fig. 1. Optimisation diagram of CL intensity of photolyzed xanthone, $C = 10^{-4}$ M vs. concentration of sodium hydroxide; best base concentration is 10^{-2} M.

were started by adding aqueous sodium hydroxide solutions (200 μl, 2 M) into the solutions of the photolyzed aromatic ketones (400 μl, 10^{-4} M). For CL measurements with hydroxyl radical (Fenton reagent) the light reactions were started by adding ferrous sulphate (100 μl, 10^{-2} M) and hydrogen peroxide solutions (100 μl, 10^{-2} M), successively, into a mixture of photolyzed ketone (400 μl, 10^{-4} M). For CL measurements with singlet oxygen, the light reactions were started on addition of hydrogen peroxide solutions (100 μl, 10^{-2} M) and sodium hypochlorite (100 μl, 10^{-2} M), successively, into a mixture of photolyzed ketones (400 μl, 10^{-4} M). For the CL measurements with the potassium superoxide radical anion, the light reactions were started by adding 200 μl saturated solution of potassium superoxide in dimethylsulphoxide (DMSO) into the photolyzed ketones (400 μl, 10^{-4} M). At this point, it should be noted that the solubility of potassium superoxide in DMSO is known to be ca. 10^{-3} M [12]. In the presence of crown ethers, the solubility of this strong oxidizing reagent can be enhanced up to 2.4×10^{-1} M [13]. The CL intensities based on the reaction mixture of photolyzed ketones are shown in Table 1. The PSCL quantitative determination of ketones was performed as follows: deaerated ketone solutions of various concentrations in DMF were placed in 5 ml square quartz cuvettes and were photolyzed with the full spectrum of a 1000 W xenon lamp for 1 min. The CL measurements were performed with 400 μl samples on addition of aqueous sodium hydroxide solutions (200 μl, 2 M). The duration of light emission was not longer than few seconds. The maximum CL intensity, in most cases, is reached in less than 0.5 s. A typical CL intensity–time diagram for photolyzed xanthone is shown in Fig. 2.

2.4. Photolysis and CL products

Photolysis of the aromatic ketones of the present work results in a complex mixture of products. The identifica-

Table 1
CL intensities (mV) of photolyzed aromatic ketones with sodium hydroxide and ROS

Number	Aromatic compounds	Photolysis time (s)	Sodium hydroxide[a] (NaOH, 2 M)	Fenton-reagent[b] (OH$^{\bullet}$)	Superoxide[c] (O$_2{}^{\bullet-}$)	Sinlet-oxygen[d] (^{1}O$_2$)
1	Anthrone	40	790	70	600	1500
2	Fluorenone	40	370	23	700	2300
3	Anthraquinone	40	360	52	630	1005
4	Dibenzosuberenone	60	6600	80	1340	2400
5	Xanthone	60	470	220	1350	1400
6	N-Methylacridone	60	3050	800	1700	3200[e]
7	Acridone	90	3450	10	290	4500[e]

[a] Photolyzed ketone (400 μl, 10^{-4} M) + aqueous sodium hydroxide solution (200 μl, 2 M).
[b] Photolyzed ketone (400 μl, 10^{-4} M) + ferrous sulphate (100 μl, 10^{-2} M) + hydrogen peroxide solutions (100 μl, 10^{-2} M).
[c] Photolyzed ketone (400 μl, 10^{-4} M) + 200 μl saturated potassium superoxide in DMSO.
[d] Photolyzed ketone (400 μl, 10^{-4} M) + hydrogen peroxide solution (100 μl, 10^{-2} M) + sodium hypochlorite (100 μl, 10^{-2} M).
[e] Measurements after dilution of the photolyzed ketone to 10^{-5} M.

tion of some photolysis products as well as CL products was performed with GC/MS, UV and fluorescence measurements and comparison with authentic samples, for example, xanthenol, fluorenol as photolysis products or xanthone, N-methylacridone, fluorenone, acridone, dibenzosuberenone, anthrone and anthraquinone as CL products.

2.4.1. Typical procedure for working out and identification of photo- and CL products with GC/MS

(a) *Photolysis products.* Deaerated solutions of aromatic ketones in DMF (5 ml, 10^{-3} M) were photolyzed until the characteristic bands in the UV spectrum disappeared. The solvent was then removed at room temperature with the aid of an oil vacuum pump; the remainder was diluted in HPLC grade dichloromethane (1 ml) and was injected in the GC/MS apparatus (10 μl). GC measurements indicated a complex mixture of products together with the dimer of DMF (N,N'-dimethyl-N,N'-diformyl-ethylenediamine), retention time at 22.9 min and coupling products of DMF with aromatic ketones as well as the reduction products of ketones (acridanol, xanthenol, fluorenol, anthranol and dibenzosuberenol) being the major products (retention times between 29 and 38 min).

(b) *CL products.* Deaerated solutions of aromatic ketones in DMF (5 ml, 10^{-3} M) were photolyzed until the characteristic bands in the UV spectrum disappeared. Aqueous sodium hydroxide (2 M, 1 ml) was added and the solvents were removed at room temperature with the aid of an oil vacuum pump and the remainder was extracted with water/chloroform, the organic phase was dried over magnesium sulphate and used for the spectroscopic measurements. Comparison of the gas chromatograms of the photolysis and CL reaction products showed that only two photoproducts corresponding to the alcohols and coupling products with the solvent–reagent DMF had been transformed back
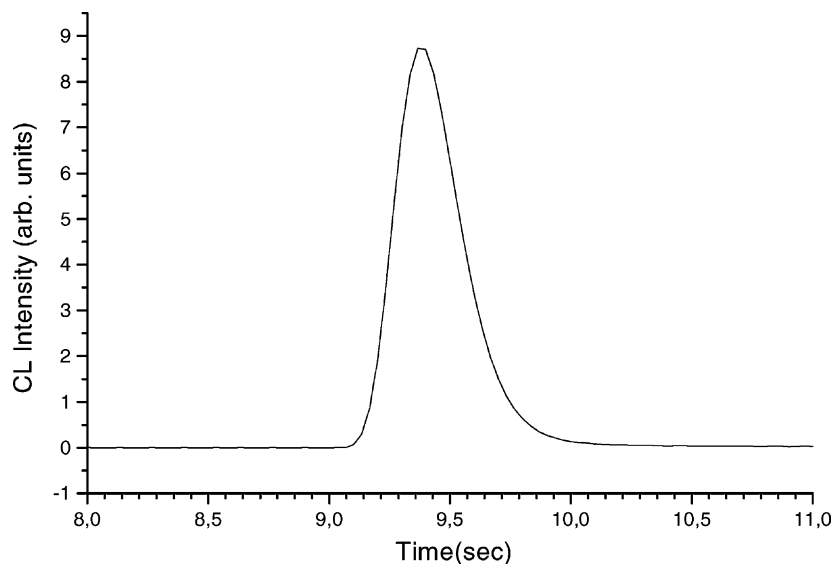

Fig. 2. Representative CL intensity–time diagram of photolyzed xanthone with sodium hydroxide. The maximum CL intensity is reached in less than 1 s.

to the initial ketones. In particular, in the case of xanthone, in the photolysis mixture, peaks with retention time at 29.6 min ($M^+ - 1$, 197, xanthenol) and 36.9 min (M^+, 269, coupling product of DMF with xanthone) disappeared and a new one with retention time at 30.3 min (M^+, 196, xanthone) appeared. Similar results were also obtained with photolyzed *N*-methylacridone and dibenzosuberenone (9-acridanol, retention time, 30.7 min, base peak at 194; the coupling product of NMA with DMF, at 35.7 min, M^+, 282 and dibenzosuberenol, with retention time at 32.4 min, M^+, 208; coupling product of dibenzosuberenone with DMF, with retention time at 36.8 min, M^+, 279). In the CL reaction mixture, the above products were transformed back to *N*-methylacridone, retention time at 33.7 min, M^+, 209 and to dibenzosuberenone (retention time 32.9 min, M^+, 206).

3. Results and discussion

Within the framework of PSCL we have earlier reported the photolysis of azaaromatics leading to reduction, disproportionation or coupling products, with the amides playing the role of solvent–reagent and the coupling product being the major product [1–4]. All efforts to isolate the photolysis products were fruitless although the products were stable in solution at room temperature. The UV and fluorescence spectra did not change after few days and the same is true for the CL profiles and efficiencies. Identification of the photoproducts and CL products was performed with the combined gas chromatography–mass spectroscopy method and shows that in all cases the major photolysis products are due to reduction (the corresponding alcohol of the aromatic ketone) and coupling of the aromatic ketones with the solvent–reagent, DMF. At this point, it should be mentioned that although the dimer products of photolyzed ketones could not be verified by GS/MS method, we believe that these compounds are produced by said reactions but are unstable on the GC column at higher temperatures. As we showed, 9,9′-dihydroxy-biacridanol, produced by a known method in our laboratory, decomposes on the GC column and gives other products with *N*-methylacridone being the major one. The photolysis reaction mixture was employed as it was for the CL reaction and the CL intensities herein reported are those of the overall process, i.e. they are based on the total light output from all photolysis products. Addition of sodium hydroxide (2 M) or oxidizing reagents to the reaction mixtures of the photolyzed ketones resulted in appreciable CL and this is shown in Table 1. As shown in Table 1, best results were obtained with sodium hydroxide and dibenzosuberenone, an olefin compound, on the lead, followed by hetero-aromatic ketones (*N*-methylacridone, xanthone). In the first case, it is difficult to explain the enhanced CL efficiency while in the case of the hetero-aromatic compounds, it is possible that the heteroatom lone pair of electrons

facilitates the peroxide decomposition and enhances the CL intensity via the CIEEL mechanism [14]. Regarding the CL mechanism, it is difficult to propose a unified mechanism due to the complexity of photoproducts. It is possible that many photoproducts contribute to the total light output. Following the initial formation of radical **2** together with DMF radicals through electron and proton transfer from DMF to excited ketone, it is expected that these reactive radical species react further after dimerization, disproportionation or coupling reactions to products, such as 1,2-dioxetanes (**A**), pinacols (**B**), linear peroxides (**C**), alcohols (**D** and **E**) (Scheme 1). Dioxetanes (**A**) are, usually, unstable at room temperature [15], give after their production spontaneous light (CL) and do not contribute to our CL measurements (PSCL). Pinacols (**B**) and linear peroxides (**C**) are, usually, stable at room temperature and give light on reaction with bases in the presence of oxygen [5,16,17]. Alcohols (**D** and **E**), which are produced after disproportionation or coupling reactions of the aromatic compounds with the solvent–reagent DMF and verified by GC/MS, react with ROS generated in situ giving the corresponding ketones and light [18,19]. All in all, regarding the CL mechanism of photolyzed ketones in DMF, it must be said that the ROS (hydroxyl radicals, superoxide anion or singlet oxygen) produced in situ from oxygen and sodium hydroxide [20–22] react rapidly with all the photoproducts mentioned above giving linear peroxides or dioxetanes, which in strong alkaline solutions decompose further, rapidly, to the starting ketones (**1**) (Scheme 1). Observations such as: (a) oxygen is required for the CL step, (b) ketones are always the end products and (c) linear peroxide decomposition fulfills the energetic requirements for CL, offer support to the mechanism of Scheme 1. This hypothesis is strengthened also by the work of Zhang and Mendenhall [23]. Regarding the CL spectroscopy, it is unfortunate that we were unable to obtain a meaningful spectrum due to the relatively low light intensities which did not allow the recording of a continuous spectrum, but mainly due to the intensity–time profiles in the form of short bursts of light. Attempts to obtain intensity–time diagrams at different emission wavelengths and plot the peaks vs. wavelength did not give a meaningful emission spectrum. The fluorescence spectrum, however, of the CL spent reaction mixture in the absence of energy transfer is usually a good approximation to the CL spectrum and this is similar with that of the initial ketone. In accordance with the mechanisms suggested above, the presence of oxygen is harmful during the photolysis step; in such a case the ratio of peaks is reversed and the CL efficiency is appreciably lowered. Most probably, the presence of oxygen in the photolysis step leads to linear peroxides whose spontaneous decomposition leads to spontaneous CL and therefore lower efficiencies in the ensuing CL step. Conversely, oxygen is necessary for the CL step, and of course DMF is a very good solvent for oxygen. At this point, it should be mentioned that the exact knowledge of the molecular structure of all the photolysis products is not critical for the use of the PSCL in
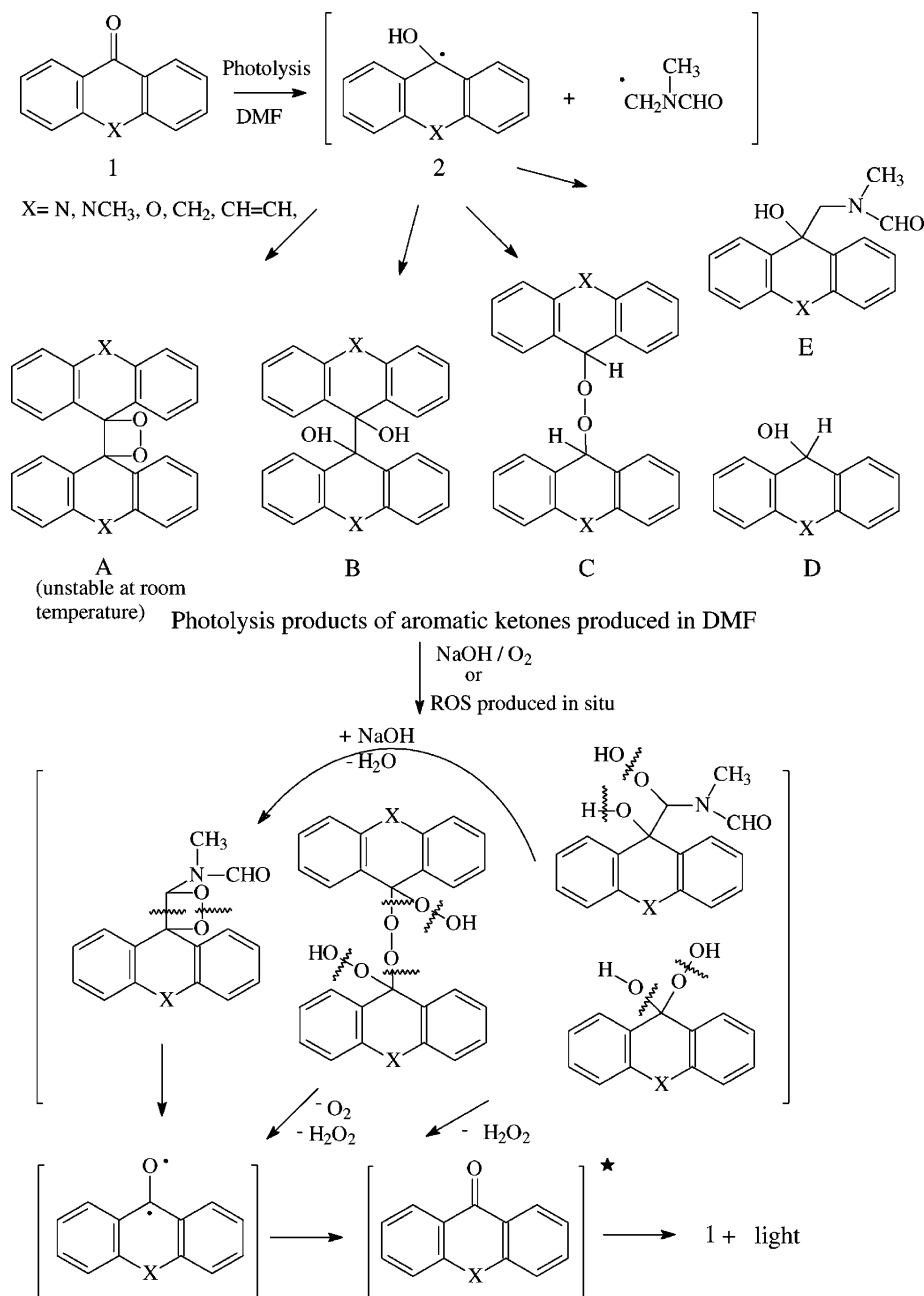
Scheme 1. Proposed PSCL reaction mechanism of photolyzed aromatic ketones with ROS.

analytical applications. Only the CL signal produced from the photoproducts is required for analytical applications and this is satisfactory in the evaluation of the total antioxidant capacity of food additives [4] or human fluids. Such a research is in our laboratory in progress and will be published shortly.

As argued above, the ketones of the present work are readily transformed to reduction and coupling products on exposure to UV light, which on reaction with hydroxides or ROS give light. The magnitude of light signals is proportional to the ketone concentrations used. It is, therefore, possible

that the PSCL reaction herein reported could be employed for the analytical applications involving these compounds. As one can see in Fig. 3, fluorenone can be determined at concentrations down to 10^{-6} M (180 ppb). The analytical parameters and linear concentration range of all photolyzed ketones are shown in Table 2. Finally, as shown in Fig. 4, the CL intensities of the photolyzed xanthone are proportional to the hydroxyl radicals used. It is therefore, possible that the PSCL reaction herein reported could be employed for quantification of this ROS at concentrations down to 10^{-4} M (1.7 ppm).

Table 2
Analytical parameters for the quantitative determination of aromatic ketones using the PSCL. For the calculation of the range the linear equation was used: $y = a + bx$, where y is the logarithm of the relative CL intensity and x the logarithm of concentration

| Number	Aromatic compounds	a	b	r	Standard deviation	Linear range (M)
1	Anthrone	5.86 ± 0.19	0.73 ± 0.04	0.9956	0.062	10^{-6}–10^{-4}
2	Fluorenone	4.19 ± 0.03	0.41 ± 0.01	0.9989	0.022	10^{-6}–10^{-3}
3	Anthraquinone	3.99 ± 0.05	0.36 ± 0.01	0.9978	0.024	5×10^{-6}–10^{-3}
4	Dibenzosuberenone	9.04 ± 0.13	1.30 ± 0.03	0.9993	0.042	10^{-5}–10^{-3}
5	Xanthone	5.45 ± 0.07	0.70 ± 0.02	0.9986	0.044	10^{-6}–10^{-3}
6	N-Methylacridone	6.83 ± 0.11	0.83 ± 0.02	0.9988	0.036	10^{-6}–10^{-4}
7	Acridone	7.06 ± 0.12	0.89 ± 0.02	0.9989	0.039	10^{-6}–10^{-4}
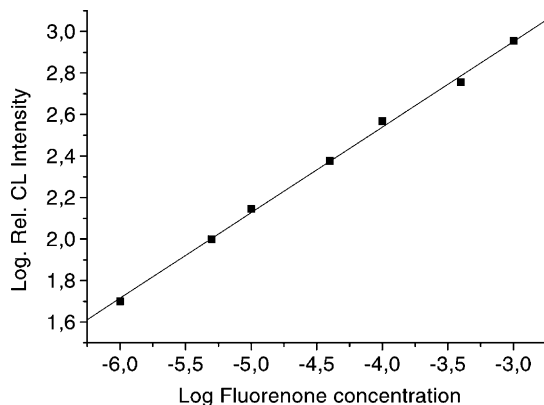
Fig. 3. Representative plot of CL intensity vs. concentration of photolyzed fluorenone (photolysis time 1 min). The parameters of the linear equation are shown in Table 2. From this diagram, fluorenone can be determined down to 180 ppb (10^{-6} M). Similar results are obtained with other aromatic ketones.
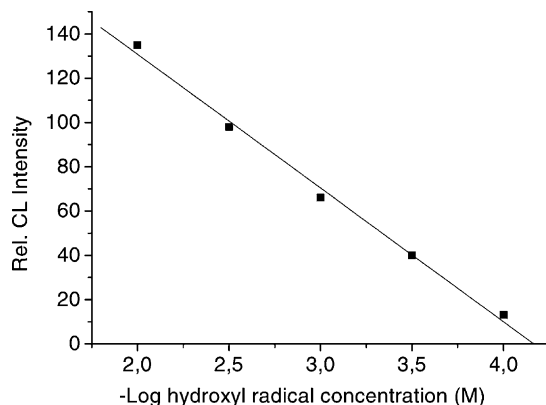

Fig. 4. Linear calibration curve, $y = a + bx$, where y is the CL intensity and x the logarithm of concentration of hydroxyl radicals for their quantitative determination from 170 to 1.7 mg/l ($a = 251.6 \pm 8.1$, $b = -60.1 \pm 2.6$, $r = -0.9971$, S.D. = 4.19).

4. Conclusions

In this work, it is shown that the light emission of photolyzed ketones with alkali hydroxides comes, generally, from superoxide anion or singlet oxygen and with lesser efficiency from hydroxyl radicals. Besides being an energy storage system in the sense that light or ionizing radiation is absorbed, stored and light is emitted at will, the PSCL reactions of the present work promise detection limits of ketones down to less than 1 ppm. Although the contribution of the hydroxyl radicals on the total light output is negligible (which could be advantageous if one is interested in other ROS), the PSCL of aromatic ketones could, eventually, be used for the detection of this ROS down to 1.7 ppm.

Acknowledgements

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