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Radiochemiluminescence of acridones and alkyl acridines

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Abstract

Acridone and 9-benzylacridine in dialkylated amides are radiolyzed to novel acridan derivatives which emit light upon addition of strong bases regenerating acridone. The chemiluminescence quantum yields are as high as 2.4×10^{-2} for acridone, while for the 9-benzylacridine 3.1×10^{-3} einstein mol^{-1}. The acridone quantum yield is higher than those of most classical chemiluminescent reactions. The radiolysis and chemiluminescence mechanisms are discussed. The radiochemiluminescence reactions constitute prospective radiation dosemeters.

Keywords: Acridone; 9-Benzylacridine; Chemiluminescence; Radiochemiluminescence

1. Introduction

We have recently observed that exposure of de-aerated acridine in *N,N*-dialkylated amides even to the diffuse light of the laboratory or to γ-irradiation results in photo- or γ-radiolysis, while addition of strong bases to said spent mixtures gives rise to very efficient chemiluminescence (CL) [1,2]. In the present work we wish to report the very strong radiochemiluminescence of acridone **1a**, *N*-methyl acridone **1b** and 9-benzylacridine **2** in dialkylated amides, such as *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), *N,N*-diethylformamide (DEF) and *N,N*-diethylacetamide (DEA) in the presence of strong bases such as sodium hydride, sodium methoxide, potassium *t*-butoxide or even sodium hydroxide (Scheme 1). It should be noted that although the CL quantum yield of 9-benzylacridinium with persulphates can be as high as 8×10^{-2} [3] the CL quantum yields herein reported are higher than those of the classical CL systems of luminol, lucigenin and lophines.

2. Experimental techniques

2.1. Reagents

Acridone and *N*-methylacridone were purchased from Aldrich and were used without further purification. 9-Benzylacridine **2** was synthesized from acridine and benzyl magnesium bromide according to known method [4,5]. The purity of all compounds used was checked by NMR, UV and fluorescence spectroscopy. The alkylated amides were purified and dried by the standard procedure [6]. Sodium hydride was purchased from Aldrich as powder and used without further purification. Sodium methoxide and potassium *t*-butoxide were purchased from Merck and used without further purification. Working solutions were freshly made, and were not employed for periods over 1 day.

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 3 nm). ^{1}H- and ^{13}C NMR spectra were measured on a Brucker AC 250 spectrometer. IR spectra were recorded with the aid of a Perkin-Elmer Model 283 spectrophotometer. Finally, 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}.

2.3. Chemiluminescence measurements

These were performed on a LKB 1250 Bio-Orbit luminometer 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 main-

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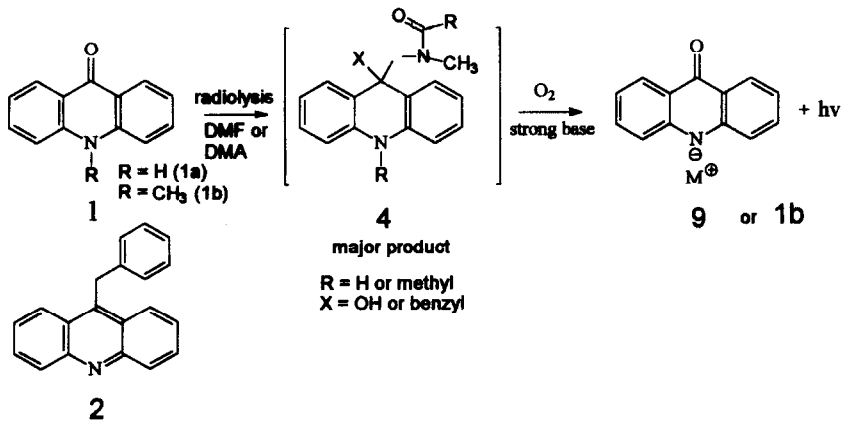


Scheme 1. Radiochemiluminescence of acridones **1a,b** and 9-benzylacridine **2** in *N,N*-dimethylamides upon the addition of strong bases.

tained at 25.0 ± 0.1°C. The light reactions were started by adding solid hydride or alcoholate or sodium hydroxide solution (10 M) into the aerated solution of the radiolyzed acridone or *N*-methylacridone (0.15 ml, 10^{-4} M) or 9-benzylacridine (1.0 ml, 10^{-4} M). The light intensity–time integrals thus obtained were compared with the Luminol Standard [7] which served as an absolute photon source under the same geometry. The quantum yields based on acridone **1** employed were over 10^{-2} einstein mol^{-1} for DMF while those based on other amides are shown in Table 1. CL quantum yields or CL intensities as a function of γ-dose from the ^{60}Co source were obtained with DMF solutions of 0.15 or 1.0 ml of radiolyzed acridone and 9-benzylacridine, respectively.

2.4. γ-Radiolysis

γ-Radiolysis experiments were performed in a ^{60}Co, 6500 Ci, Gamma Chamber (4000 A, Isotope Group, Bhaba Atomic Research Centre, Trombay, India). The dose rate was determined with Fricke's dosemeter [8] and was found equal to 35.12 Gy min^{-1}. Doses were calculated from Dose = $n \times 10.94 + 35.12 \times$ time(min) Gy, n, being the number of consecutive irradiations. Radiolysis was considered complete on disappearance of the acridone's absorption at ca. 400 nm or at 366 nm for 9-benzylacridine (ca. 40 min, $C = 10^{-4}$ M). Further radiolysis resulted in destruction of the radiochemical products absorbing at 324 and 336 nm for acridones and at 294 nm for 9-benzylacridine accompanied by a dramatic reduction in CL. Eventually, after radiolysis of acridone for 120 min, only one absorption peak remains at 287 nm.

3. Results and discussion

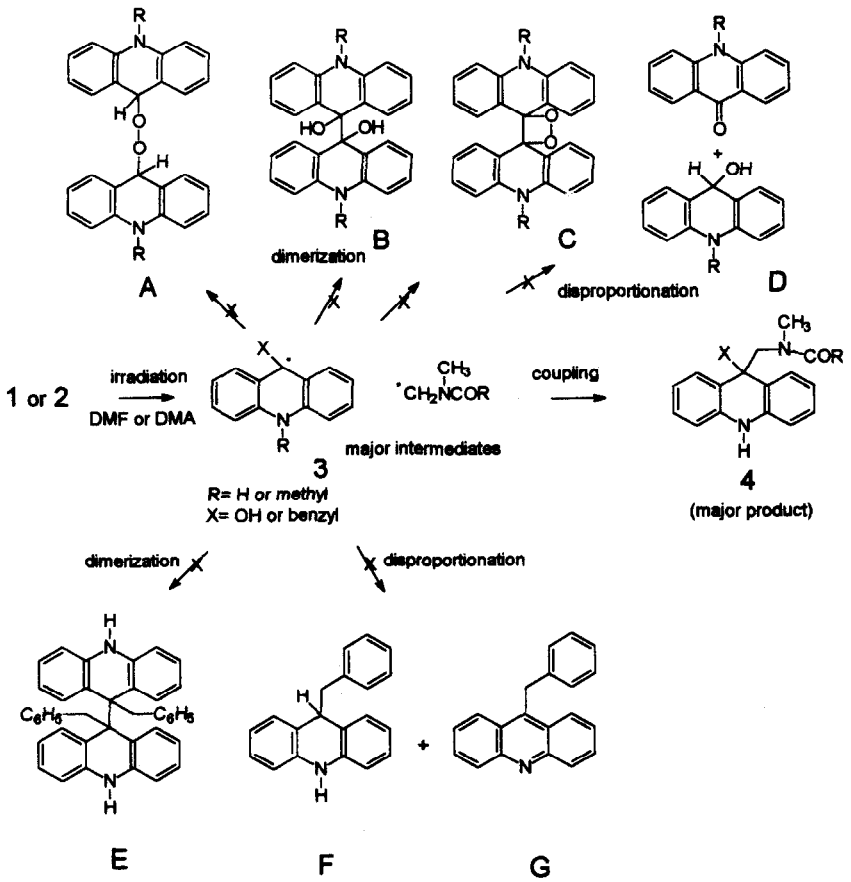
3.1. The radiolysis step

Photoreaction of acridines has been a well-studied subject. 9,10-Dihydroacridine is a major photoproduct of the dilute solutions of acridine in most solvents, while 9,9′,10,10′-tetrahydro-9,9′-biacridinyl is also produced at higher acridine concentrations [9–11]. Photochemical reactions of acridones have also been reported, but most of them have dealt with photodimerization (cycloaddition reactions leading to cyclic dioxo-compounds, such as 1.2-dioxetanes, Suzuki et al. [12], pinacols, Kawata et al. [13], peroxides, Stauff and Stark [14]

Table 1
Radiochemiluminescence quantum yields of acridone **1a**, *N*-methyl-acridone **1b** and 9-benzylacridine **2** ($C = 10^{-4}$ M) in amide solvents

Substance	Solvent	Radio-CL quantum yields (einstein mol^{-1})			
		NaH	CH_3ONa	KOH[a]	$^1O_2$[b]
Acridone[ac]	DMF	2.42×10^{-2}	1.09×10^{-2}	1.22×10^{-2}	1.23×10^{-6}
	DMA	1.10×10^{-2}	1.12×10^{-2}	1.18×10^{-2}	2.55×10^{-6}
N-methyl-acridone[abd]	DMF	2.46×10^{-5}	2.46×10^{-5}	2.29×10^{-5}	7.0×10^{-6}
	DMA	2.58×10^{-5}	3.20×10^{-5}	2.27×10^{-5}	8.2×10^{-6}
9-Benzyl-acridine[abde]	DMF	2.32×10^{-3}	1.15×10^{-3}	1.04×10^{-3}	1.32×10^{-6}
	DMA	3.82×10^{-3}	4.52×10^{-3}	3.44×10^{-3}	7.65×10^{-6}

[a] 10 N KOH, 200 μl.
[b] NaOCl (200 μl, 3%) + H_2O_2 (200 μl, 3%).
[c] 150 μl radiolyzed solution.
[d] 1.0 ml radiolyzed solution.
[e] ϕ_{CL}, ca. 2×10^{-4} einstein mol^{-1}, before radiolysis, (NaH or CH_3ONa, DMF).



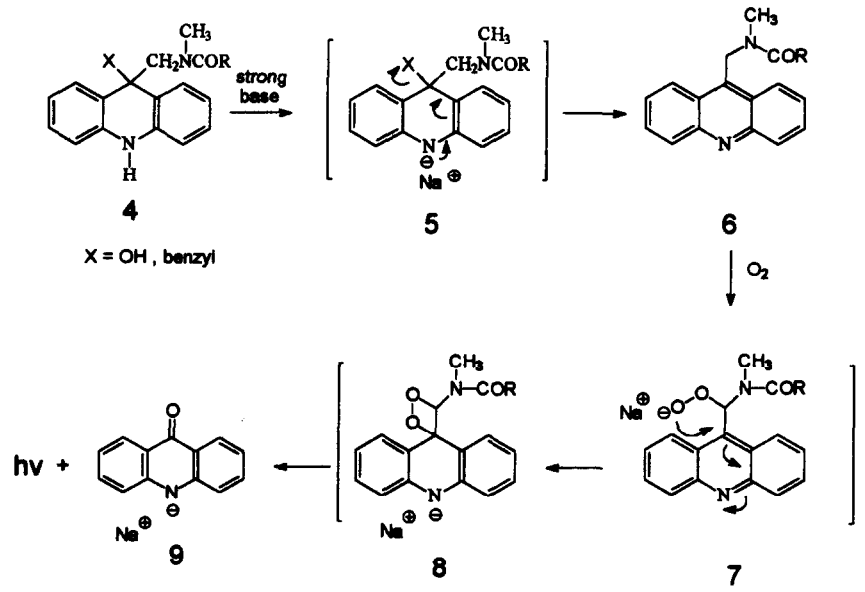
Scheme 2. Radiolysis products of acridones **1a,b** and 9-benzylacridine **2**.

or disproportionation reactions [15,16] (Scheme 2)) but, to our knowledge, nothing has been reported on the radiolysis of acridines or acridones. Such reactions lead to coupling products with the amide as solvent–reagent.

The radiolysis products of both acridone and 9-benzylacridine, although stable in solution could not be isolated by all forms of chromatography, fractional crystallization or sublimation. Following the initial formation of radical **3** together with DMF radicals [17] (Scheme 2) and by analogy with acridine [1,2] where the DMF adduct has been isolated and identified, we expect coupling products **4** to be the major products of the radiolysis step and the CL precursors. In any case it cannot be dioxetan **C** as this compound, also intermediate of the lucigenin CL reaction is unstable above −78°C. Pinacols **B** on the other hand, suggested by Niizuma and Kawata [15] for the photolysis of acridones should be also ruled out. Indeed, 9,9-dihydroxyacridan **B**, similar to that obtained from lucigenin on addition of sodium hydroxide and prepared by ourselves, in DMF has an entirely different UV spectrum. (cf. diol **B** 362 and 395 nm—radiolyzed solution 335 and 347 nm, respectively). Furthermore, diol **B** emitted no light upon addition of strong bases whereas our radiolysis product is strongly CL. Linear peroxide **A** proposed by Stauff and Stark [14] for the photolysis of *N*-methylacridone in acetone, should be also ruled out as Stauff's and Stark's peroxide is CL upon addition of secondary amines while the radiolysis product of the present work is not. Yet another possibility, i.e., disproportionation of acridan radical **3** to acridanol **D** was not verified as the singlet expected at 4 ppm for the proton at the 9 position of **D** was not present in the ^{1}H NMR spectrum of the condensed radiolyzed solution.

As argued above, our previous results with acridine [1,2] advocate for coupling product **4** as radical $^{\bullet}CH_2N(CH_3)CHO$ is the main product of the DMF radiolysis [17]. In addition the ^{13}C NMR spectrum of radiolyzed 9-benzylacridine shows the characteristic peaks of DMF at 63.7 (NCH_2), 36.4 (NCH_3) and 163.3 ppm (NCHO), not present in the ^{13}C NMR spectrum of condensed radiolyzed DMF, thus further strengthening the coupling hypothesis. It should be noted, however, that all attempts to isolate **4** chromatographically resulted in decompositions yielding mainly **6** (Scheme 3) as shown by ^{13}C NMR, while heating the radiolyzed solution to 90°C for 60 min gives back the original acridone. The presence of the strong base might not really be necessary in the step **4** → **6**, as **4** decomposes during thin layer chromatography to **6** in the absence of base. The possible catalytic effect, however, of the TLC bed on this oxidation, not present in our reaction in solution should also be considered before rejecting the requirement for bases in this step.

Spectroscopic monitoring of the reactions showed that in common with DMF solutions, radiochemical reactions of



Scheme 3. Proposed CL reaction mechanism of acridans **4** with strong bases in the presence of oxygen.

acridones in DMA, DEF and DEA proceeded the same way (similar UV, fluorescence and NMR spectra), yet isolation of the products proved difficult. Conversely, radiolysis of acridone in other aprotic solvents such as acetone, diethylether, pyridine, acetonitrile, dimethylsulphoxide and chloroform did not lead to analogous products.

3.2. The chemiluminescence step

The CL of acridone **1** and 9-benzylacridine **2** after radiolysis on addition of strong bases required the presence of atmospheric oxygen. The influence of the solvent or the basicity of the bases on the CL quantum yields are shown in Table 2. It is interesting to note that CL is far more efficient in dimethylamides (DMF, DMA) than in diethylamides (DEF, DEA). The duration of light emission as reflected on the CL intensity–time diagrams (not shown) depends on the basicity of the strong base and the solvent. It ranges from 2 min (sodium hydride, DMF) up to 15 min (sodium methoxide, DMA). If necessary the said strong bases can be replaced by 10 N aqueous sodium or potassium hydroxides with only 10–50% loss of CL. It was also observed that although nonradiolyzed acridone and *N*-methylacridone are not CL under the conditions of this work, 9-benzylacridine before radiolysis exhibited CL one order of magnitude lower than that after radiolysis. Also, the products of both acridone and *N*-methylacridone radiolysis are CL in reaction with singlet oxygen with a quantum yield of the order of 10^{-6} einstein mol^{-1} (Table 1). Regarding the mechanism of the CL step a most interesting observation is that replacement of the nitrogen proton by a methyl group ruins CL (Table 1), (cf. acridone vs. *N*-methylacridone) indicating that the lone pair of electrons on nitrogen is crucial for the CL step.

Deprotonated acridone **9** was the primary emitter of the light reactions in all cases and this is shown in Fig. 1. Here, typically, the CL spectrum is depicted together with the flu-

Table 2
Absorption and fluorescence data of nonradiolyzed and radiolyzed acridones **1a,b** and 9-benzylacridine **2**

Spectroscopic and CL data (in DMF, DMA)		Nonradiolyzed	Radiolyzed
Acridone	UV, λ (nm)	396, 377, 360, 308, 295	324, 336
	Isosbestic point	353	
	Fluorescence, λ (nm)	415, 435	420
	CL spectrum	472, 498, 531*	484, 502, 528
N-methylacridone	UV, λ (nm)	401, 382, 308, 295, 278, 270	346, 332, 324
	Isosbestic point	360	
	Fluorescence, λ (nm)	418, 436	418
	CL spectrum	425	
9-Benzylacridine	UV, λ (nm)	348, 361, 386	294
	Isosbestic point	332	
	Fluorescence, λ (nm)	408, 422	372
	CL spectrum	482, 507, 530	482, 507, 530

*λ_{max} of authentic sodium acridonyl.

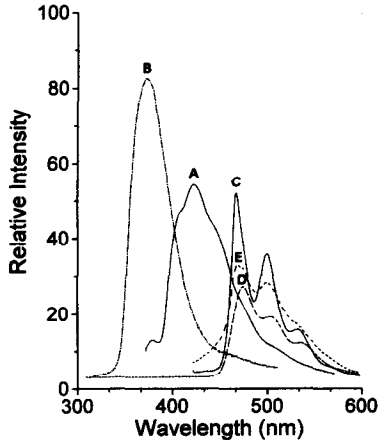


Fig. 1. Emission spectra in DMF. Spectrum A, fluorescence of 9-benzylacridine before radiolysis; spectrum B, fluorescence of the reaction mixture after radiolysis; spectrum C, fluorescence of sodium acridonyl; spectrum D, CL spectrum of radiolyzed acridone upon addition of NaH; spectrum E, fluorescence spectrum of the CL spent reaction mixture.

orescence of radiolyzed and nonradiolyzed 9-benzylacridine **2** and the fluorescence spectrum of the CL spent reaction mixture; the fluorescence spectrum of an authentic sample of sodium acridonyl **9** (λ_{max} 472, 498 and 531 nm) is also shown; similar results were obtained with acridone. Considering that (a) oxygen is required for the CL step, (b) ionization of the nitrogen proton should be facile, (c) acridone is in all cases the end product and primary emitter, (d) dioxetan decomposition of **8** fulfils the energetic requirements for CL and (e) the mechanisms so far proposed for 9-substituted acridines [2,18,19], the mechanism of Scheme 3 is now advanced.

As mentioned above acridan **4a** of the present work is readily obtained from acridone on exposure to γ-radiation and its reaction with strong bases is CL with an efficiency higher than those of luminol and lucigenin. It is, therefore, possible that the radiochemiluminescence reactions herein reported can be employed as radiation dosemeters. Indeed, radiolysis causes a decline in the absorption peak of acridone at 396 nm and an increase in the region around 324 nm with an isosbestic point at 356 nm. After 40 min the absorption of acridone was virtually zero and this of the radiochemical product **4a** showed ε_{max} 7420 at λ_{max} 324 nm (10^{-4} M in DMF) (Fig. 2).

In Fig. 3, the absorbance difference of acridone **2** is plotted vs irradiation dose. Acridone is destroyed and as the γ-irradiation dose increases, higher absorbance difference values due to decreased acridone concentration are obtained. This type of plot has the prospects of becoming a γ-radiation dosemeter and possibly a dosemeter for internal irradiation of solutions by radioactive isotopes in amide solvents in the region 100–1100 Gy.

Furthermore, the product in the γ-radiolysis spent mixture, in reaction with sodium hydride and other strong bases gives rise to CL whose intensity–time integrals are, as expected, a function of radiation absorbed. The light integrals or light

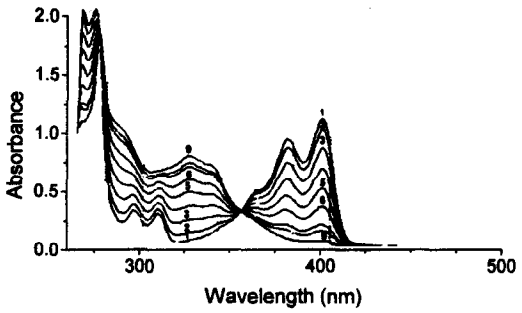


Fig. 2. Absorption spectra of acridone **1a** upon radiolysis in degassed DMF. Spectrum 1, before radiolysis; radiolysis time interval 5 min; $C = 10^{-4}$ M.

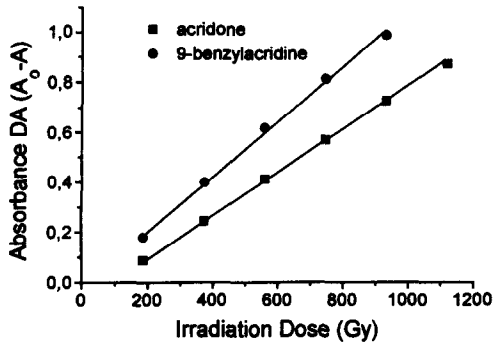


Fig. 3. Plots of absorption (expressed as absorbance difference from the value of nonradiolyzed to radiolyzed acridone or benzylacridine) of acridone **1a** or 9-benzylacridine **2** in DMF vs. absorbed dose; $C = 10^{-4}$ M.

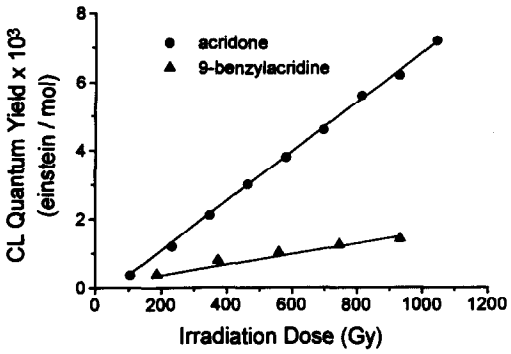


Fig. 4. CL quantum yields of radiolyzed acridone or 9-benzylacridine ($C = 10^{-4}$ M) in DMF upon addition of NaH, plotted vs. absorbed γ-ray dose.

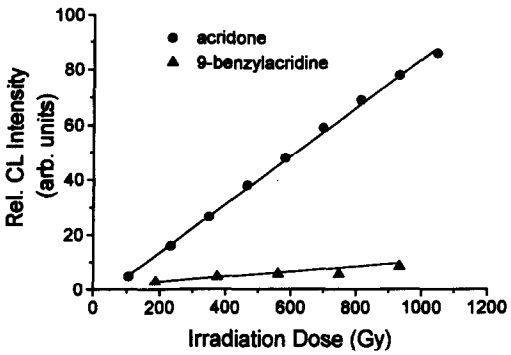


Fig. 5. CL light intensities of radiolyzed acridone or 9-benzylacridine ($C = 10^{-4}$ M) in DMF upon addition of NaH, plotted vs. absorbed γ-ray dose.

intensities thus obtained are directly proportional to the dose absorbed and this is shown in Figs. 4 and 5, where the dose was determined with the aid of Fricke's actinometer [8].

Although under our conditions, linearity exists only for the first 40 min of irradiation, this plot shows that this reaction can also 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 100–1100 Gy. It should be noted, however, that with 10^{-3} M *N*-methylacridone concentration and singlet oxygen [NaOCl (100 μl, 3%) + H_2O_2 (100 μl, 3%)] the plot of relative CL intensity vs. irradiation dose is linear between 2000 and 14000 Gy.

4. Conclusions

The present radiochemiluminescent system possesses the following characteristics: (a) It is cyclic in the sense that acridone is radiolyzed and acridone is regenerated as the end CL product. (b) The CL quantum yields are higher than those of classical systems such as lophines, luminol and lucigenin, not exceeding 10^{-2} einstein mol^{-1}. The said quantum yields are in fact even higher as they are the end result of a two step process. (c) Plots of acridone absorption vs. γ-irradiation dose as well as plots of CL intensity–time integrals vs. γ-irradiation dose indicate that both types of plots can be employed as radiation dosemeters.

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