

## Sensitized chemiluminescence in micellar mixtures of phthalhydrazide and selected dyes

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Received 18 March 2004; accepted 28 April 2004

Available online 20 July 2004

### Abstract

A simplified quantitative model of chemiluminescence intramolecular sensitization is proposed, based on the analytical solution of Förster's energy transfer function for donor–acceptor pairs residing on micellar spherical surface. To test the usability of the model, the intramolecular sensitization of phthalhydrazide chemiluminescent oxidation by energy transfer to several xanthene as well as non-xanthene dyes has been investigated. The reactions were initiated using three different initiation systems: copper sulphate–hydrogen peroxide system, sodium hypochlorite and hydrogen peroxide–sodium hypochlorite. In this work, we bring an experimental evidence that different transfer mechanisms operate, if phthalhydrazide-sensitized chemiluminescence reactions are initiated by copper sulphate–hydrogen peroxide, sodium hypochlorite or sodium hypochlorite–hydrogen peroxide. Only the latter system is selective enough to oxidize phthalic hydrazide much more rapidly than dyes employed as acceptors. This fact enables the application of the proposed model to achieve the quantitative description of the sensitization process.

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**Keywords:** Phthalhydrazide; Sensitized chemiluminescence; Energy transfer; CTA micelles

### 1. Introduction

The sensitization of luminol chemiluminescence by the addition of fluorescein was one of the very first observations of energy transfer phenomenon in chemiluminescence. It was reported by Plotnikov as early as in 1938 [1] and called chemifluorescence, further investigations were done by Tamamushi [2]. Micellar effects on the sensitization process were first reported by Lasovsky and Grambal [3,4], the topic was reviewed by Hinze et al. [5]. The peculiar features of the sensitization process are not completely understood and novel reports on this topic still appear from time to time [6–8]. Unlike luminol, the oxidation of which results in singlet excited state of aminophthalate, the oxidation of its parent compound phthalhydrazide yields excited phthalate triplet. It has been reported that excitation energy of triplet phthalate can be transferred to various substrates such as xanthene or phthalocyanine dyes in a micellar system [9] or to rare earth cations in solid matrices [10].

Roberts and White [11] investigated chemiluminescence energy transfer from phthalhydrazide to various fluorescing moieties, directly attached to phthalhydrazide benzene ring via a methylene bridge. They found that triplet–singlet energy transfer of mixed exchange and Förster nature occurs in most of these conjugates. Luminol intramolecularly sensitized chemiluminescence (ISCL) is thought to proceed completely via Förster energy transfer mechanism [12]. From the facts given above, it appears that Förster energy transfer creates a natural framework for the description of phthalhydrazide ISCL. Förster-type energy transfer conducted in micellar solution has been a subject of many theoretical studies, e.g. [13–17]. The common feature of these is that they consider micelles as monodisperse hard spheres; treatments of stationary case (the diffusion of donor and acceptor on micellar surface is much slower than the energy transfer event) as well as of general case (comparable rates of diffusion and transfer) are available as the results of Monte Carlo simulations [13,14] or in the form of explicit analytical expressions [15–17]. However, these works were aimed to explain decay laws of donors or acceptors in time-resolved fluorescence experiments and therefore, they deal with the rates of energy transfer rather than with transfer efficiencies. To our

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best knowledge, the only paper in which energy transfer efficiency is evaluated is [18], where a transfer function for randomly distributed donor and acceptor pairs residing inside a sphere is obtained by Monte Carlo simulation. In this work, we bring the analytical solution of a stationary case intracellular energy-transfer efficiency for donor and acceptor located on a surface of a sphere, which we believe is relevant for the description of phthalhydrazide ISCL, initiated by  $\text{NaClO-H}_2\text{O}_2$ . We also report briefly our finding that a change in transfer mechanism occurs if chemiluminescence reactions of phthalic hydrazide sensitized by xanthene dyes are initiated by alkaline  $\text{CuSO}_4\text{-H}_2\text{O}_2$ .

## 2. Experimental

### 2.1. Chemicals

Phthalhydrazide (PH, 2,3-dihydro-1,4-phthalazinedione), hydrogen peroxide (Sigma), fluorescein disodium salt, 4',5'-dichlorofluorescein (Fluka), eosin Y, eosin B, erythrosin (Lachema Brno, Czech Republic), sodium 9,10-dimethoxy-2-anthracene sulphonate (DAS, Molecular Probes), chloroaluminium phthalocyanine disulphonate ( $\text{AlPcS}_2$ ) was a gift from Dr. Rakusan, VUOCH Institute, Rybitvi, Czech Republic. Hexadecyltrimethylammonium hydroxide (CTAOH) was prepared from CTAB on Dowex 1X2 anionic exchanger, while sodium hypochlorite was prepared by saturation of cooled NaOH solution by chlorine gas.

### 2.2. Instruments

BioOrbit 1250 luminometer (Finland) was used for chemiluminescence measurements except for the measurements with chloroaluminium phthalocyanine disulphonate, which were performed using homemade luminometer equipped with cooled R649 Hamamatsu (Japan) photomultiplier sensitive in the red region of spectrum. All chemiluminescence data were corrected with respect to the spectral responses of photomultipliers. Fluorescence measurements were done using a Hitachi F4500 spectrofluorometer, and absorption spectra were obtained on Beckman DU 7500 spectrometer (USA). Data handling methods: Statistica version 5.1. software package was used for the statistical evaluation of data.

### 2.3. Chemiluminescence measurements

A series of chemiluminescence measurements have been made depending on the oxidation system used. For reactions using sodium hypochlorite as oxidation reagent, 250  $\mu\text{l}$  of aqueous solution of this compound ( $2 \times 10^{-5}$  M) were added into 1750  $\mu\text{l}$  solution containing phthalhydrazide ( $2.5 \times 10^{-6}$  M), hydrogen peroxide ( $1 \times 10^{-3}$  M) and different concentrations of CTAOH ( $2\text{--}8 \times 10^{-3}$  M). The same volumes and concentrations of the reagents were used for chemiluminescence measurements in the presence

of dyes. For reactions in which copper sulphate was used as cooxidant, 250  $\mu\text{l}$  of hydrogen peroxide ( $3 \times 10^{-3}$  M) were added into 1750  $\mu\text{l}$  solution containing copper sulphate ( $4 \times 10^{-4}$  M), CTAB ( $2\text{--}8 \times 10^{-3}$  M) and  $2 \times 10^{-6}$  M phthalhydrazide and  $1.2 \times 10^{-4}$  M dye.

### 2.4. Fluorescence quantum yield determination

Fluorescence quantum yields of dyes used in this work in CTAOH solutions were calculated by comparison with that of fluorescein sodium salt aqueous solution in the presence of 0.01 M NaOH, which is equal to 0.92 [19].

## 3. Results and discussion

Phthalhydrazide and dyes employed in this work are bulky counteranions to  $\text{CTA}^+$  cationic micelles, therefore strongly bound in the micellar pseudophase, the solubilization site being the micellar-Stern region. Binding constant for fluorescein in CTAB micelles is 492 [20], a value of 155 is reported for luminol in CTAC micelles [21]. Since phthalhydrazide is less polar than luminol, it is safe to expect even higher value of binding constant for phthalhydrazide. The oxidation of cyclic phthalhydrazide results in a very weak emission from the triplet state of the phthalate anion as can be seen from the coincidence between its chemiluminescence spectrum and the emission spectrum of phthalate (not shown). In photoluminescence experiment, phthalate is excited at 320 nm from  $^1\text{A}_{1g}$  to  $\text{B}_{2u}$  state ( $n \rightarrow \pi^*$  transition), undergoes fast intersystem crossing and phosphoresce at 425 nm in both aqueous (phosphate buffer, pH 7.6) and CTAOH micellar solutions. The quantum yield is approximately  $10^{-4}$  (0.00012, estimated by comparison to fluorescein-disodium salt standard) and is essentially the same for both the above mentioned environments. Low luminescence quantum yield of phthalate precludes low values of critical Förster distances (around 1 nm), the usually reported lower limit for the usability of Förster equations [22]. Assuming overall quenching constant of phthalic acid phosphorescence in air-saturated micellar solution to be around  $10^{10}$ , the phosphorescence lifetime should be subnanosecond. In this case, a stationary energy transfer (i.e. transfer process not affected by mutual diffusion of donor and acceptor) on a spherical surface would apply. The overall efficiency of intracellular energy transfer  $\Phi_{\text{ET}}$  depends on the following two factors: (1) occupation statistics—i.e., on the probability that donor molecules share the same micelle with acceptor molecule(s)—the fraction of “active micelles”  $\alpha_{\text{active}}$  and (2) on the average energy transfer efficiency  $\Phi_{\text{ET}}^{\text{M}}$  in an “active micelle”:

$$\Phi_{\text{ET}} = \Phi_{\text{ET}}^{\text{M}} \alpha_{\text{active}} \quad (1)$$

If the experiments are performed at low PH concentrations ( $c_{\text{PH}} \ll c_{\text{M}}$ ,  $c_{\text{M}}$  denotes the concentration of micelles), only free micelles, or micelles occupied by a single PH molecule are present in the reaction mixture, the fraction of micelles

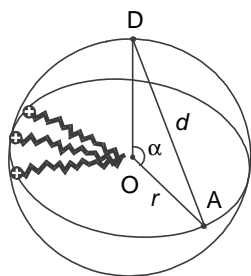


Fig. 1. Model of spherical micelle. D, A are donor and acceptor molecules separated by the distance  $d$ ,  $\alpha$  is the angle between donor, center of the micelle O and acceptor,  $r$  is micellar radius.

occupied by pH is given by  $c_{\text{pH}}/c_{\text{M}}$ . Energy transfer is then governed by dye–micellar distribution only, instead of the ternary PH–dye–micelle complex formation. For diluted micellar region, the Poisson-distribution model works well and is generally accepted throughout the literature [23,24]. The fraction of micelles  $\alpha_n$  containing  $n$  molecules of dye F is given by Eq. (2):

$$\alpha_n = [MF_n]/c_{\text{M}} = \langle (n)n! \rangle \exp(-\langle n \rangle) \quad (2)$$

$\langle n \rangle$  is the average number of dye molecules in the micelle  $\langle n \rangle = c_{\text{F}}/c_{\text{M}}$ . The concentration of micelles is given by  $c_{\text{M}} = (c_{\text{S}} - \text{cmc})/N_{\text{AG}}$ , where  $c_{\text{S}}$  is the concentration of surfactant, cmc its critical micellar concentration and  $N_{\text{AG}}$  mean aggregation number denoting the number of surfactant molecules which form a micelle. The constant  $\Phi_{\text{ET}}^{\text{M}}$  in Eq. (1) is an average energy transfer efficiency in an “active micelle” and its evaluation is necessary if enhancement factors are to be predicted. We derived a transfer function for a statistical assembly of spherical micelles, donor and acceptor pairs located on micellar surfaces with random mutual distances (Fig. 1). The efficiency of Förster ET between the donor D and acceptor A separated by a distance  $d$  situated on a surface of spherical micelle having diameter  $r$  is:

$$\Phi_{\text{ET}}(\text{one micelle}) = \frac{1}{1 + (d/R_0)^6} = \frac{1}{1 + \{(2^6 r^6 \sin^6(\alpha/2))/R_0^6\}} \quad (3)$$

(meaning of  $\alpha$  is obvious from Fig. 1,  $R_0$  is the critical Förster distance given by:

$$R_0^6 = \frac{9000(\ln 10)}{128\pi^5 N} \left( \frac{\Phi_{\text{D}}^0 K^2}{n^4} \right) \int_0^\infty F_{\text{D}}(\tilde{\nu}) \varepsilon_{\text{A}}(\tilde{\nu}) \frac{d\tilde{\nu}}{\tilde{\nu}^4} \quad (4)$$

where  $N$  is Avogadro’s number,  $n$  refractive index of the medium,  $\Phi_{\text{D}}^0$  is the quantum yield of emission of donor in the absence of acceptor,  $K$  is the orientational factor,  $F_{\text{D}}$  is the corrected fluorescence intensity of the donor with the total intensity normalized to unity,  $\varepsilon_{\text{A}}$  is the acceptor extinction coefficient,  $\tilde{\nu}$  is the wave number. A discussion on orientational factor can be found elsewhere [25], the value of  $K^2$  for aqueous solution is 0.75, for micellar solutions the

value  $K^2 = 0.475$  can be found in the literature. The probability of finding an acceptor at a distance  $d$  from donor depends on angle  $\alpha$  (it is proportional to the circumferences of micellar crosssections perpendicular to DOA planes, these circumferences are  $2\pi r \sin \alpha$ ). Corresponding probability density is  $2\pi r \sin \alpha / \int 2\pi r \sin \alpha d\alpha = -\sin \alpha/2$ . For a statistical assembly of micelles, donor and acceptor pairs residing on micellar surfaces with random mutual distances  $d$  the following integral has to be evaluated ( $\alpha' = \alpha/2$ ):

$$\begin{aligned} \Phi_{\text{ET}}^{\text{M}} &= -\frac{1}{2} \int_0^\pi \frac{\sin \alpha}{1 + \{(2^6 r^6 \sin^6(\alpha/2))/R_0^6\}} d\alpha \\ &= \int_0^{\pi/2} \frac{\sin \alpha' \cos \alpha'}{1 + \{(2^6 r^6 \sin^6 \alpha')/R_0^6\}} d\alpha' \end{aligned} \quad (5)$$

Integration of the Eq. (5) gives Eq. (6):

$$\begin{aligned} \Phi_{\text{ET}}^{\text{M}} &= \frac{R_0^2}{2r^2} \left( \frac{1}{3} \ln \left( \frac{4r^2}{R_0^2} + 1 \right) - \frac{1}{3} \ln \left( \frac{4r^2}{R_0^2} + \sqrt{3} \frac{2r}{R_0} + 1 \right) \right. \\ &\quad - \frac{1}{6} \ln \left( \frac{4r^2}{R_0^2} - \sqrt{3} \frac{2r}{R_0} + 1 \right) \\ &\quad + \frac{\sqrt{3}}{6} \arctg \left( \frac{4r}{R_0} + \sqrt{3} \right) \\ &\quad \left. + \frac{\sqrt{3}}{6} \arctg \left( \frac{4r}{R_0} - \sqrt{3} \right) + \frac{\sqrt{3}}{3} \arctg \left( \sqrt{3} \right) \right) \end{aligned} \quad (6)$$

The course of the transfer function is depicted on the Fig. 2, transfer function for fixed distance case (which would correspond to donor situated in the centre of a micelle and acceptor on its surface or vice versa) is included for

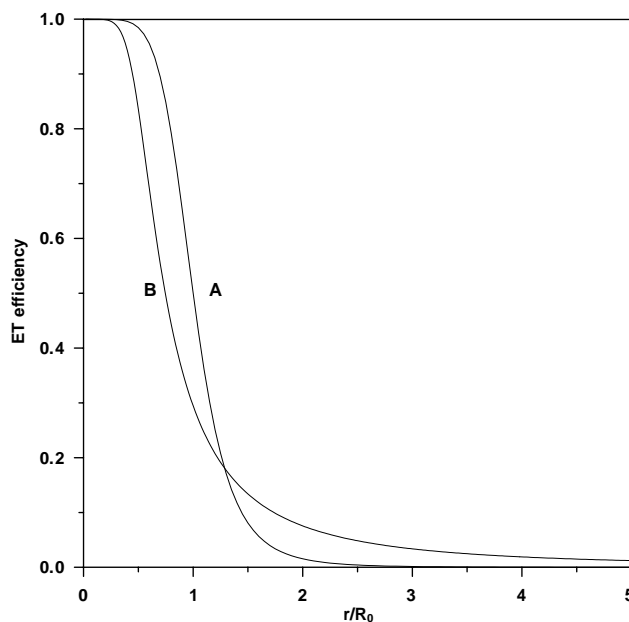


Fig. 2. Förster energy transfer functions according to Eq. (3) (A) and Eq. (6) (B).

comparison. To verify our considerations experimentally, we carried out a series of sensitized chemiluminescence experiments with phthalhydrazide in micellar solutions of CTAOH. In our efforts, we encountered a problem of judicious selection of the CL reaction-initiation system, selective enough to oxidize phthalic hydrazide only, but not the dyes employed as acceptors. We found that hydrogen peroxide decomposition catalysed by transition metal cations (typically  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  or  $\text{Co}^{3+}$ ;  $\text{Cu}^{2+}$  being among the most widely used ones), often employed in luminol chemiluminescence, does not fulfill this requirement nor does sodium hypochlorite. However, the reaction of phthalhydrazide with sodium hypochlorite and hydrogen peroxide is much faster than the corresponding reaction with all dyes employed in this work.

### 3.1. The initiation of ISCL by alkaline copper sulphate–hydrogen peroxide system

The reactions of radicals generated by  $\text{Cu}^{2+}$ – $\text{H}_2\text{O}_2$  initiation system with phthalhydrazide and dyes in micellar solution of CTAOH results in chemiluminescence (Fig. 3). The sensitization process is strongly pH dependent and corresponds to the dyes' chemiluminescence pH profiles rather than phthalhydrazide pH profile (Fig. 3). Therefore, it appears that concurrent reactions of dyes with radicals generated by the initiation system give rise to efficient energy transfer chemiluminescence process with quantum yield approaching unity in an "active micelle". A detailed account on PH-sensitized chemiluminescence initiated by mixtures

of transition metal cations and  $\text{H}_2\text{O}_2$  is in the preparation for separate publication.

### 3.2. The initiation of ISCL by sodium hypochlorite

The initiation by NaClO is the simplest way to achieve phthalhydrazide ISCL in regard of the reaction mixture complexity. Unfortunately we have found that NaClO is decomposed by most of the dyes used. These concurrent reactions were not observed with DAS, the effect of fluorescein and eosin Y was moderate, but eosin B, erythrosin and dichlorofluorescein completely prevented NaClO reacting with PH. Chemiluminescence is destroyed in the presence of additives such as a buffer or salt. The reason for this is the fact that NaClO is only weakly bound to micelles [21]. Since the reactions of dyes with hypochlorite are virtually non-chemiluminescent, ISCL initiated by hypochlorite is characterised by the highest ratio between ISCL intensities and background chemiluminescence intensities caused by weak chemiluminescence of dyes. The  $I_{\text{SCL}}/I_{\text{Flu}} = 2$  occurs at  $c_{\text{pH}}$  as low as  $5 \times 10^{-9}$  M, a value that could be interesting from the analytical viewpoint.

### 3.3. The initiation of ISCL by sodium hypochlorite–hydrogen peroxide system

If excess hydrogen peroxide is present in the reaction mixture, the addition of hypochlorite results in singlet oxygen generation, which reacts preferentially with phthalhydrazide. The kinetics of sensitized chemiluminescence

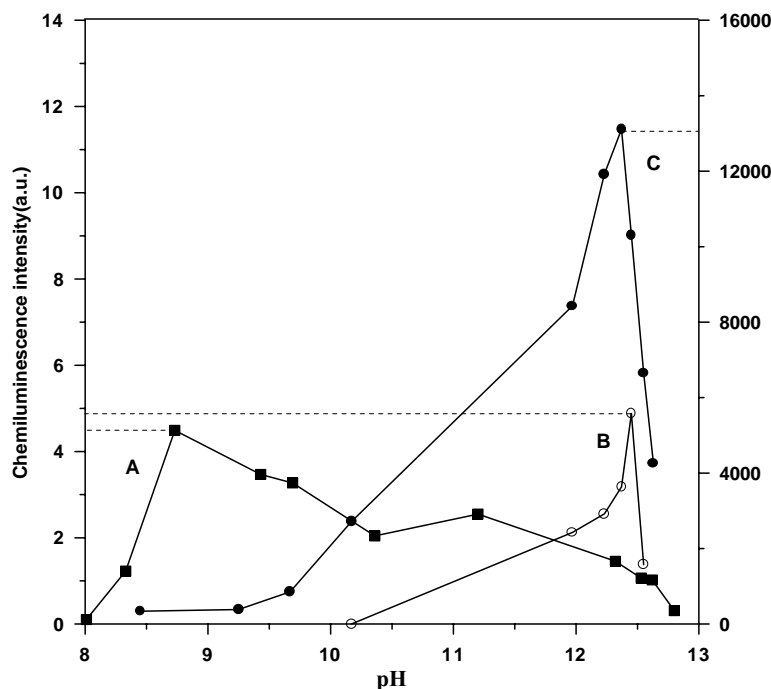


Fig. 3. Dependence of chemiluminescence intensity of phthalhydrazide, fluorescein and mixture of fluorescein and phthalhydrazide vs. pH; 250  $\mu\text{l}$  of  $\text{H}_2\text{O}_2$  ( $3 \times 10^{-3}$  M) were added to 1750  $\mu\text{l}$  solutions containing  $4 \times 10^{-4}$  M  $\text{CuSO}_4$ ,  $1.6 \times 10^{-3}$  M CTAH and  $2 \times 10^{-6}$  M phthalhydrazide (A) or  $1.2 \times 10^{-4}$  M fluorescein (B) or a mixture of phthalhydrazide and fluorescein (C) in concentrations given before.

are identical for all dyes within used concentration range and match the time profile of PH chemiluminescence (Fig. 4). In this figure, the chemiluminescence intensity data of each curve were divided by the curve's maximum CL intensity (normalized CL intensity =  $I_{CL}(t)/I_{max\ CL}$ ). Normalized CL intensities obtained this way enable rapid assessment, whether the presence of the acceptor dye in the reaction mixture influences the kinetics of the ISCL reaction or not. Fig. 5 shows the dependencies of maximum ISCL intensities on fluorescein concentration for several concentrations of CTAOH. Similar dependencies are observed also for other dyes used. Their courses are consistent with the idea that sensitization is effective only in micelles occupied by one molecule of PH and one dye molecule ("active micelle"). The higher occupied micelles are inefficient due to quenching, the same observation is reported in [12] for luminol chemiluminescence sensitized by fluorescein. Fitting experimental data with corresponding Eq. (7):

$$\begin{aligned} \Phi_{ET} &= \Phi_{ET}^M \frac{c_M(c_{pH}/c_M)(c_{dye}/c_M) e^{-c_{dye}/c_M}}{c_{PH}} \\ &= \Phi_{ET}^M \frac{c_{dye}}{c_M} e^{-c_{dye}/c_M} \end{aligned} \quad (7)$$

(Poissonian distribution for 1:1 micelle:dye complex formation) allows for the determination of the concentrations of micelles and consequently their aggregation numbers ( $N_{AG}$ ).

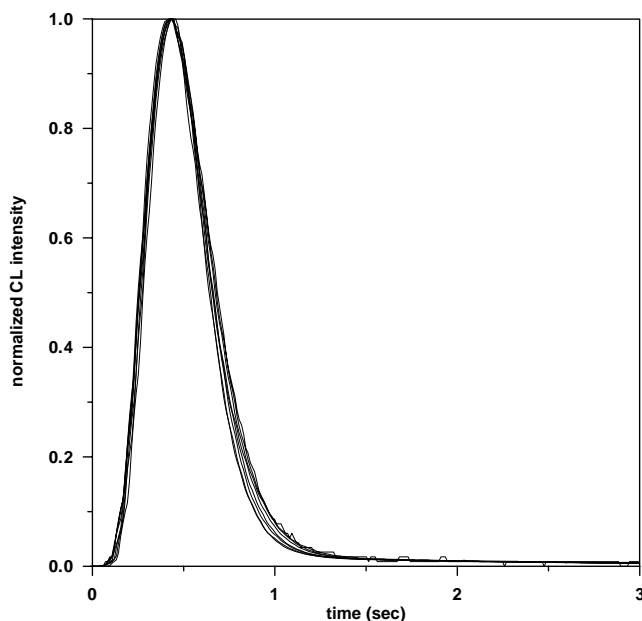


Fig. 4. Kinetics of sensitized chemiluminescence light signals of phthalhydrazide in the presence of different fluorescein concentrations ( $2.5 \times 10^{-6}$  M PH,  $1 \times 10^{-5}$  M NaClO,  $1 \times 10^{-3}$  M  $H_2O_2$ , 4 mM CTAOH,  $0.5 \times 10^{-4}$  M fluorescein).

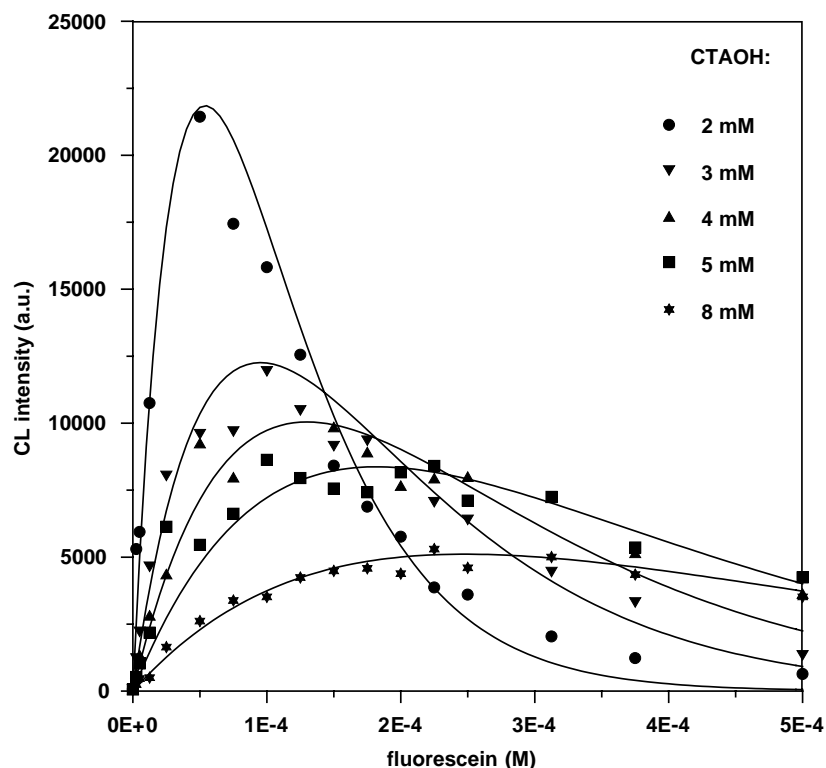


Fig. 5. Chemiluminescence intensities of phthalhydrazide in CTAC micellar solutions as a function of fluorescein concentration ( $2.5 \times 10^{-6}$  M PH,  $2.0 \times 10^{-5}$  M NaClO,  $1.0 \times 10^{-3}$  M  $H_2O_2$  and 2–8 mM CTAOH).

Table 1

Determination of aggregation numbers and micelle concentrations by non-linear regressions of sensitized chemiluminescence of phthalhydrazide in the presence of dyes

Surfactant concentration (mM)	Micelle concentration $c_M$ (M) <sup>a</sup>	Aggregation number <sup>b</sup> $N_{AG}$	Coefficient of determination
Fluorescein			
2	$5.4 \times 10^{-5}$	28	0.98
3	$9.5 \times 10^{-5}$	26	0.97
4	$1.3 \times 10^{-4}$	24	0.96
5	$1.8 \times 10^{-4}$	25	0.93
8	$2.5 \times 10^{-4}$	30	0.99
Eosin Y			
2	$4.4 \times 10^{-5}$	34	0.92
3	$9.7 \times 10^{-5}$	26	0.99
4	$1.6 \times 10^{-4}$	22	0.99
5	$2.2 \times 10^{-4}$	20	0.99
8	$3.8 \times 10^{-4}$	20	0.99
4',5'-dichlorofluorescein			
2	$1.5 \times 10^{-5}$	101	0.97
3	$2.4 \times 10^{-5}$	102	0.97
4	$3.3 \times 10^{-5}$	107	0.98
5	$4.2 \times 10^{-5}$	107	0.96
8	$5.9 \times 10^{-5}$	127	0.96
Erythrosin <sup>c</sup>			
2	$2.6 \times 10^{-5}$	58	0.90
3	$3.9 \times 10^{-5}$	64	0.85
Eosin B			
2	$8.7 \times 10^{-6}$	172	0.96
3	$2 \times 10^{-5}$	125	0.98
4	$3.5 \times 10^{-5}$	100	0.95
5	$5.5 \times 10^{-5}$	81	0.91
8	$8 \times 10^{-5}$	93	0.92
Chloroaluminium phthalocyanine tetrasulphonate			
2	$5.5 \times 10^{-6}$	275	0.99
3	$1.1 \times 10^{-5}$	227	0.93
4	$1.5 \times 10^{-5}$	230	0.93
5	$2 \times 10^{-5}$	225	0.95
8	$2.8 \times 10^{-5}$	250	0.80
Sodium 9,10-dimethoxy-2-anthracene sulphonate <sup>d</sup>			
2	$7.7 \times 10^{-5}$	19	0.60
3	$1.5 \times 10^{-4}$	17	0.94
4	$2.4 \times 10^{-4}$	15	0.98
5	$3.4 \times 10^{-4}$	13	0.99
8	$5.7 \times 10^{-4}$	13	0.99

<sup>a</sup> Calculated after fitting the experimental data in Eq. (7).

<sup>b</sup> Calculated using the equation:  $N_{AG} = (c_s - \text{cmc})/c_M$ ; where cmc of CTAOH is  $5 \times 10^{-4}$  M and  $c_s$  and  $c_M$  are given.

<sup>c</sup> Data for 4, 5 and 8 mM CTAOH were statistically insignificant.

<sup>d</sup> After approximately 30 s from the addition of DAS solution into solution of CTAOH a microcrystalline phase is formed as a precipitate. This is in agreement with low  $N_{AG}$  values observed.

Determined aggregation numbers are of stable values for used CTAOH concentrations but differ for each dye, indicating the individual character of micellization (Table 1). It appears that in diluted micellar region fluorescein, eosin Y and DAS form aggregates with  $N_{AG} \approx 30$ , while dichlorofluorescein and other dyes tend to form larger ones. The enhancement factor (EF) is given by (Eq. (8)) (donor emission being omitted):

$$EF = \frac{\Phi_F \Phi_{ET}}{\Phi_{PA}} \quad (8)$$

where  $\Phi_F$  is the fluorescence quantum yield of acceptor dye,  $\Phi_{PA}$  is the quantum yield of phthalate luminescence ( $\Phi_{PA} = 0.00012$ ). Data are summarized in Table 2. Experimental values of enhancement factors agree well with theory except for chloroaluminium phthalocyanine disulphonate, where exchange interactions probably predominate. The best agreement between experimental and theoretical enhancement factors is achieved in 2–3 mM CTAOH concentrations, in more concentrated CTAOH, the observed enhancement is approximately 20% lower than predicted one.



Table 2  
Parameters of phthalhydrazide intracellular sensitization

Dye	$J$ (cm <sup>6</sup> mol <sup>-1</sup> ) <sup>a</sup>	$R_0$ (nm) <sup>b</sup>	$N_{AG}$ <sup>c</sup>	$r$ (nm) <sup>d</sup>	$\Phi_{ET}^{Me}$ <sup>e</sup>	$\Phi_F$ <sup>f</sup>	EF <sup>g</sup> (theoretical)	EF <sup>h</sup> (found)
Fluorescein	$6.6 \times 10^{-14}$	0.98	27	1.4	0.15	0.62	285	320
Eosin Y	$3.8 \times 10^{-14}$	0.89	24	1.4	0.14	0.51	234	228
Eosin B	$4.0 \times 10^{-14}$	0.90	100	2.1	0.06	0.12	22	20
Erythrosin	$5.6 \times 10^{-14}$	0.95	60	1.8	0.08	0.09	22	18
4',5'-Dichlorofluorescein	$5.2 \times 10^{-14}$	0.94	109	2.2	0.06	0.58	106	91
DAS	$7.7 \times 10^{-15}$	0.68	15	1.2	0.10	0.45	137	140
AlPcS <sub>4</sub>	$1.5 \times 10^{-14}$	1.5	241	2.9	0.08	0.16	39	80

<sup>a</sup> Determined as integral of spectral overlap between the donor emission and the acceptor absorption.

<sup>b</sup> Calculated from Eq. 4; where  $k^2 = 0.475$  [15],  $\Phi_D = 0.00012$ ,  $n = 1.334$  [12].

<sup>c</sup> Average values given in Table 1.

<sup>d</sup> Determined as  $V_{micelle} = N_{AG} (27.4 + 26.9 (n_c - 1)) (\text{\AA}^3)$ ,  $V_{micelle} = (4/3)\pi r^3$  [26].

<sup>e</sup> Determined from equation 6,  $R_0$  and  $r$  are values given in table.

<sup>f</sup> Fluorescence quantum yield determined at 4 mM CTAOH.

<sup>g</sup> Calculated from Eq. (8), where  $\Phi_{ET} = 0.368 \cdot \Phi_{ET}^M$ ,  $\Phi_F$  and  $\Phi_{ET}^M$  are values given in table, 0.368 is the fraction of micelles occupied by single dye molecule at  $c_{dye}/c_{micelle} = 1$ ,  $\Phi_{PA} = 0.00012$ .

<sup>h</sup> Determined at  $c_{dye}/c_{micelle} = 1$  for 2 mM CTAOH.

## 4. Conclusions

Analytical solution of Förster energy-transfer efficiency for donor–acceptor pair residing on the spherical surface is derived in this work. In combination with donor–acceptor occupational statistics, the model can be used for quantitative description of chemiluminescence intracellular sensitization process. Phthalhydrazide chemiluminescence sensitized by several xanthene as well as non-xanthene dyes in the CTAOH micellar solutions, initiated by the addition of sodium hypochlorite to the reaction mixture containing excess hydrogen peroxide was used to verify the usability of theory.

## Acknowledgements

This work was supported by MSM 153100007 grant from the Ministry of Education of the Czech Republic. The authors wish also to thank the General Secretaries of Research and Technology of Greece and Czech Republic for funding part of this project.

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