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Photoinduced chemiluminescence of pharmaceuticals

B. Gómez-Taylor [a], M. Palomeque [b], J.V. García Mateo [a,*], J. Martínez Calatayud [c]

[a] *Departamento de Ciencias Químicas, Universidad Cardenal Herrera CEU, Moncada (Valencia), Spain*
[b] *Departamento de Química, Universidad Nacional del Sur, Bahía Blanca, Argentina*
[c] *Departamento de Química Analítica, Universidad de Valencia, Valencia, Spain*

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Abstract

A screening test for the forward development of chemiluminescence systems able to determine pharmaceutical compounds is reported. The test is based on the on-line photodegradation of the drugs by using a photoreactor consisting of 697 cm × 0.5 mm PTFE tubing helically coiled around an 8 W low-pressure mercury lamp. Photodegraded pharmaceuticals are detected by direct chemiluminescence of the resulting photofragments and their subsequent reaction with potassium permanganate in sulphuric acid medium as oxidant. The screening comprised 97 compounds with different molecular structures and relevant members of the most important families of pharmaceuticals are tested (amino acids, carboxylic acids, nitrocompounds, phenyl-alkyl and aromatic amines, sulphonic acid amides, polycarbocyclics, monocyclic N-containing heterocyclics, bicyclic N-containing heterocyclics, tricyclic N-containing heterocyclics, N-S containing heterocyclics. . .). Due to the relevant influence of the medium for the photodegradation a wide range of pH's and buffer solutions were studied. The proposed strategy (photoinduced chemiluminescence, Ph-CL) allows the development of systems for the determination of many pharmaceuticals which do not present "native" chemiluminescence (e.g. chloramphenicol, dextromethorpham, riboflavin, ephedrine, piperazinamide, chlotrimazole, theophylline. . .). Moreover, Ph-CL allows to increase the sensitivity of chemiluminescence procedures based on direct chemiluminescence detection (e.g. sulphonamides, thiazides, nicontinamide, nortryptiline, levamisole, phenylbarbituric acid. . .).

Keywords: Chemiluminescence; Photolysis; Pharmaceuticals; FIA

1. Introduction

Liquid phase chemiluminescence is very attractive as a means of highly sensitivity detection of substances presenting pharmaceutical, clinical or environmental interest [1–4], but its development has been limited by the scarcity of molecules that are strongly chemiluminescent in solution.

The tandem photodegradation-chemiluminescence detection offers an interesting strategy to increase the number of compounds to be determined by chemiluminescence thanks to the chemiluminescent properties of the resulting photofragments. Recently, photochemical reaction and chemiluminescence detection have been applied to the determination of aromatic amines [5], carbofuran [6], Vitamin K3 [7], nitrate[8,9], lactate [10] and generation of singlet molecular oxygen [11,12].

To the category of sources of discreet spectrum and low power, low-pressure mercury lamps are presented as excellent due to their attractive analytical features such as simplicity, low cost and sensitivity. The main line of the spectrum is 254 nm and there are another lines between 300 and 600 nm. These spectral lines are useful for many photoreactions, because most of organic compounds (pharmaceuticals included) present absorption bands in this spectral range. The use of germicidal lamps (low-pressure mercury lamps) has proved to be an efficient strategy to promote the photolysis with high selectivity and to allow the implementation of reactions involving complex mechanisms in inorganic [13,14], environmental [15,16] and pharmaceutical analysis [17–19].

Although a wide range of different chemical reactions have been described in photochemistry, on-line photochemical reactions in chemiluminescence analysis have been described only for a relatively small number of substances, almost all of them leading to a fluorescent or electrochemically active product after irradiation [20,21]. Extending this principle of photochemical

* Corresponding author. Tel.: +34 6 136 90 00; fax: +34 96 139 52 72.
E-mail address: jvgarcia@uch.ceu.es (J.V. García Mateo).

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reaction to chemiluminescent detection systems photoinduced chemiluminescence (Ph-CL), we have investigated whether drugs of various molecular structures are modified in their chemiluminescent properties by on-line irradiation in order to search for new and more sensitive chemiluminescent systems involving pharmaceuticals.

In this work, a screening test for the forward development of chemiluminescence systems suitable for analysis of pharmaceuticals is proposed. The proposed strategy comprises a photochemical reaction by using a photoreactor (a coiled PTFE tubing around a low-pressure mercury lamp for germicidal use) and chemiluminescence detection of photoirradiated pharmaceuticals. Several photodegradation media were examined because the relevance of pH on the photodegradation's pathway.

2. Experimental

2.1. Reagents

All reagents were analytically pure unless stated otherwise and prepared in deionized water (18 MΩ cm) by using a Sybron/Barnstead Nanopure II water purification system provided with a fiber filter of 0.2 μm pore-size. Aqueous solutions of pharmaceuticals were prepared by dissolving the drug in deionized water. Cysteine (Fluka), methionine (Scharlau). Other L-amino acids were glutamic acid, arginine (both from Merck), lysine, phenylalanine, tyrosine, threonine, isoleucine, histidine, glutamine, serine, asparagine, tryptophan, alanine, proline, isoproline, valine (all from Guinama), glycine and leucine (from U.C.B.). Other tested drugs were from Guinama. $KMnO_4$ and H_2SO_4 employed in the oxidant solution were from Probus. Acetic acid, sodium acetate, NaH_2PO_4, Na_2HPO_4, glycine, borax, NH_4Cl, NH_3, H_2SO_4 and NaOH (all Probus) were used for preparing the media of photodegradation. All solution were deareated by bubbling hellium during 15 min.

2.2. Apparatus

The flow manifold consisted of a PTFE coil of 0.5 mm i.d., a Rheodyne (Cotati, CA, USA) Model 5041 injection valve, a Gilson (Worthington, OH, USA) Minipuls 2 peristaltic pump provided with pump Tygon tubing from Omnifit and an 8 W low-pressure mercury lamp (Zalux) for germicidal use. The flow cell was a flat-spiral quartz tube of 1 mm i.d. and 3 cm total diameter backed by a mirror for maximum light collection. The photodetector package was a P30CWAD5F-29 type 9125 photomultiplier tube (PMT) supplied by Electron Tubes operating at 1280 V and was located in a laboratory-made light-tight box. The output was fed to a computer equipped with a counter-timer, also supplied by Electron Tubes.

2.3. Flow injection assembly

The proposed FIA-manifold is depicted in Fig. 1 Aqueous solutions of tested compounds at a 5×10^{-4} mol l^{-1} concentration and medium of photodegradation were forced to pass at a flow-rate of 1 ml min^{-1} through channels 1 and 2, respectively.

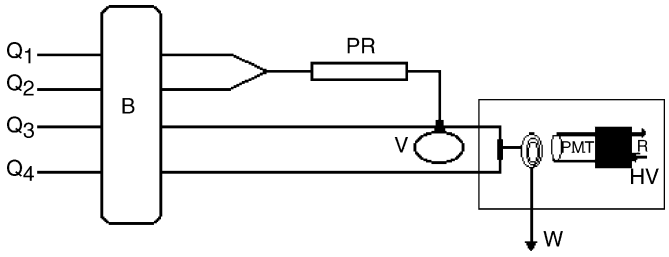

Fig. 1. Flow injection assembly for photoinduced chemiluminescence of pharmaceuticals. Q_1, sample solution (aqueous solution of drug 5×10^{-4} mol l^{-1}), 1.0 ml min^{-1}; Q_2, medium of photodegradation, 1.0 ml min^{-1}; Q_3, carrier solution (deionized water), 6.0 ml min^{-1}; Q_4, oxidant solution $KMnO_4$, 1.6 ml min^{-1} in 2 mol l^{-1} H_2SO_4; V, sample loop, 524 μl; B, peristaltic pump; PR, photoreactor (697 cm long × 0.5 mm i.d. piece of PTFE tubing coiled around an 8 W low-pressure mercury lamp); PMT, photomultiplier tube and W, waste.

The mixture was photoirradiated in a photoreactor consisting of 697 cm × 0.5 mm PTFE tubing helically coiled around an 8 W low-pressure mercury lamp. Then 524 μl of irradiated sample were injected into a pure water carrier stream flowing at 6 ml min^{-1}. The photodegraded drug reacted with the oxidant solution (2×10^{-4} mol l^{-1} $KMnO_4$ in 2 mol l^{-1} sulphuric acid) flowing at 1.6 ml min^{-1}. The resulting mixture reached the flat spiral quartz flow cell and the total chemiluminescence emission was detected by a photomultiplier tube working at 1280 V.

2.4. Procedure

All pharmaceuticals were tested with the lamp OFF and ON. A wide range of pH was studied employing the following solutions: H_2SO_4 10^{-3} mol l^{-1}, H_2O, acetic acid/sodium acetate (pH 4.5), NaH_2PO_4/Na_2HPO_4 (pH 7.1), glycine buffer (pH 8.6), borax buffer (pH 9), NH_4^+/NH_3 (pH 10), NaOH 10^{-3} mol l^{-1} and 0.5 mol l^{-1} (all buffers in concentration 0.02 mol l^{-1}). Chloramphenicol [18] was employed as a test substance and prior to a new screening session the system was checked with the aid of a 5×10^{-4} mol l^{-1} aqueous solution of chloramphenicol and glycine buffer as medium of photodegradation.

3. Results and discussion

3.1. Selected chemical and physico-chemical parameters

The criteria for selecting the suitable chemical and physicochemical variables were forced by the compromise between chemiluminescence intensity and sample throughput. Preliminary studies reported by authors [18,19], and employing chloramphenicol and sulphamethoxazole as test substances showed the viability of low-pressure mercury lamps and short irradiation times for an effective degradation of the drug and a sensitive chemiluminescent detection.

3.1.1. Irradiation time

The time of exposure to UV light was examined by changing the flow-rate of the irradiated solution stream. The solution of the drug was irradiated during 45 s, considering the volume of the reactor (697 cm length × 0.5 mm i.d.) and the flow-rate at

which sample and medium of photodegradation were pumped (2 ml min^{-1}).

3.1.2. Carrier and oxidant flow-rates

Due to the fast kinetics of the chemiluminescent reaction, the carrier flow-rate was fixed at 6 ml min^{-1} and the oxidant flow-rate was 1.6 ml min^{-1}. These values provided the highest chemiluminescent emission for test compounds [19] and fulfilled the following requirements: maximum volume and shortest residential time of the analyte-oxidant bolus in the flow system, effectiveness by mixing the degraded drug and the oxidant solution in the quartz spiral flow cell, and low dilution of the inserted sample.

3.1.3. Dissolved oxygen

Dissolved oxygen can determine the formation of radical oxygen species (ROS species) during the photodegradation process. ROS species can strongly influence the mechanism of the chemiluminescent reaction. Considering the potential influence of small changes of this parameter on the chemiluminescence response, and the necessity of performing the screening in a reproducible way to compare the results from different drugs; carrier, oxidant and drug solutions were deaerated by bubbling hellium during 15 min.

3.1.4. Temperature

The screening was performed at room temperature. Due to the type and power of the UV source (low-pressure mercury lamp, 8 W), and also the short irradiation time, it was not necessary to immerse the channel containing the photoirradiated solutions into a thermostated cooling bath.

3.1.5. Selected oxidant

Acidic permanganate system gives rise to light emission for many compounds [22,23] and was selected for the present screening. In fact, it is presented as the most efficient oxidant for direct liquid phase chemiluminescent processes. A search employing the Analytical Abstract database (1980–2004) and the keywords chemiluminescence and the oxidants $KMnO_4$, Ce(IV), $Fe(CN)_6^3$, *N*-bromosuccinimide yielded as conclusions that 42% of the published papers used potassium permanganate as strong oxidant. The reason of this behaviour is associated to the mechanism of chemiluminescence generation by potassium permanganate. Several authors [24,25] have contributed to explain it. According to Barnett, it seems the emitter responsible for acidic potassium permanganate chemiluminescence is an excited manganese(II) species of unknown constitution. This hypothesis seems to be verified by enhancing effect of polyphosphates on the chemiluminescent emission using KMO_4 as oxidant. The excellent behaviour of potassium permanganate associated to direct chemiluminescence procedures should be due to an unusual case of phosphorescence at room temperature. The species (drugs employed in the screening) reacting with the oxidant should contribute to stabilize the excited manganese(II) emitter.

On the other hand, the behaviour of permanganate requires special care in selecting its concentration. The signal shows a parabolic profile increasing sharply with concentration up to a maximum value beyond which it decreases abruptly. Results obtained by authors in previous works employing KMO_4 as oxidant [18,19,24] revealed this trend. Attending to the robustness of the procedure, the 2×10^{-4} mol l^{-1} $KMnO_4$ was selected for the screening.

3.2. Tested pharmaceuticals

All drugs were prepared at the same molar concentration (5×10^{-4} mol l^{-1}) so that a comparison between the analytical signals could be established. In the screening, 97 compounds with different molecular structures and relevant members of the most important families of pharmaceuticals (amino acids, carboxylic acids, nitrocompounds, phenyl-alkyl and aromatic amines, sulphonic acid amides, polycarbocyclics, monocyclic N-containing heterocyclics, bicyclic N-containing heterocyclics, tricyclic N-containing heterocyclics and N-S containing heterocyclics) were tested. The Ph-CL behaviour of two classical and very sensitive chemiluminescence systems, e.g. DNPO (a bis-dinitrophenyloxalate) and luminol (a cyclic carbonyl hydrazide) were also tested.

Due to the important influence of the medium of photodegradation a wide range of pH's and buffer solutions were studied. Pharmaceuticals were tested at nine different pH values over the range 3–13, with the lamp OFF (native chemiluminescence) and ON (Ph-CL) on each one.

The results of the screening are summarized in Tables 1–5, in which the analytical signals with lamp OFF (in counts) and the increase (+) or decrease (−) caused by the UV irradiation are shown. The drugs were classified attending to molecular structure's criteria.

3.3. Photoinduced chemiluminescence of pharmaceuticals

As it was suspected, potassium permanganate was an effective oxidant for direct liquid phase chemiluminescence. Only dextromethorphan, ephedrine, chloramphenicol, piperazinamide, chlotrimazol, saccharine and a group of non-aromatic amino acids were not chemiluminescent in the tested media and without UV-irradiation. All the other drugs exhibited native chelimunimescence over the studied pH. Nevertheless, most of the drugs yielded a weak chemiluminescent response with lamp OFF. In fact, 41 compounds (43%) presented values <100 counts in all media.

In general, a significant increase in the chemiluminescent emission intensity was observed with lamp ON [I(ON)/I(OFF) ratios] for reference compounds were: lysine (17.3), tyrosine (45.8), norepinephrine (10.8), tetracycline (10.2), emetine (10.4), quinine (31.6), 4-aminobenzoic acid (15.1) and salicylamide (22.4). Only leucine, lidocaine, moroxydine and inositol yielded values <100 counts in all media; and 61 compounds (63.5%) were clearly chemiluminescent (>100 counts) in all tested media after irradiation.

Photodegradation with low-pressure mercury lamps and chemiluminescent detection has a strong dependence with the photodegradation medium. In general, a relevant increase

Table 1
Ph-CL of amino acids and sulphonic acid amides

Drug	H_2SO_4 0.001 M OFF/ON	H_2O OFF/ON	pH 4.5 OFF/ON	pH 7.1 OFF/ON	pH 8.6 OFF/ON	pH 9 OFF/ON	pH 10 OFF/ON	NaOH 0.001 M OFF/ON	NaOH 0.5 OFF/ON
Amino acids									
Lysine	147/+427	115/+109	–/+121	–/+191	–/+140	–/+223	–/+128	–/+96	160/+2617
Histidine	18/+45	16/+78	–/+40	–/+45	6/+84	8/+73	6/+38	5/+45	81/+261
Tyrosine	19/+442	–/+461	20/+441	7/+550	16/+716	25/+977	22/+901	29/+671	48/+1336
Phenylalanine	–/+103	–/+119	–/+175	–/+143	–/+83	–/+175	–/+143	–/+146	24/+350
Leucine	8/+37	5/+30	8/+17	6/+26	5/+29	6/+31	5/+9	5/+41	26/+48
Methionine	5/+47	12/+39	6/+71	5/+60	5/+52	12/+39	10/+36	12/+39	13/+872
Tryptophan	132/+273	117/+233	132/+166	132/+195	120/+297	123/+309	123/+280	146/+233	408/+802
Cysteine	136/+209	114/+227	114/+318	159/+47	136/+148	57/+170	60/+150	227/+568	239/+4022
Sulphonamides									
Sulphamethoxazole	70/+1041	35/+935	49/+688	35/+494	42/+512	46/+476	52/+424	64/+476	141/+706
Sulphacetamide	279/+338	281/+13	155/+409	126/+157	151/+148	109/+233	126/+155	128/+225	109/+237
Sulphadimidine	56/+567	49/+260	33/+531	38/+396	77/+267	17/+254	31/+202	46/+179	98/+344
Sulphaguanidine	52/+212	65/+188	62/+230	59/+219	32/+181	60/+212	23/+214	55/+209	42/+252
Sulphamerazine	88/+423	95/+391	62/+523	76/+448	101/+288	96/+392	74/+406	74/+406	70/+797
Sulphamethoxypyridazine	40/+355	45/+340	50/+324	42/+305	56/+316	46/+272	29/+242	46/+309	54/+234
Sulphathiazole	35/+445	36/+433	38/+414	47/+317	57/+278	44/+279	44/+208	52/+303	243/+192
Sulphanilamide	63/+599	69/+205	85/+230	69/+125	86/+165	82/+171	85/+158	87/+194	221/+194

The analytical signals with lamp OFF (in counts) and the increase (+) or decrease (−) caused by the UV irradiation (lamp ON) are shown. (−) Not chemiluminescent.

of the emission intensity was observed in strong alkaline medium (NaOH 0.5 mol l^{-1}). Fifty-four (64%) of the CL compounds with lamp ON presented the maximum increase in this medium. This percentage was 100% for the amino acids family. Sulphamethoxazole, sulphacetamide, sulphadimidine, sulphamethoxypyridazine, Vitamin B1, dextromethrorphan, proflavin, nortryptiline, bis-dinitrophenyloxalate and benzocaine exhibited the best Ph-CL behaviour for 10^{-3} mol l^{-1} H_2SO_4 as medium of photodegradation. Slightly acidic or alkaline media were not so effective.

Attending to the observed chemiluminometric response, pharmaceuticals can be divided into five groups: (a) compounds which do not present chemiluminescence with lamp OFF and ON; (b) compounds which decrease the CL with lamp ON; (c) a group presenting not relevant increase on the CL output with lamp ON; (d) clearly CL-pharmaceuticals which increased dramatically the CL-response with lamp ON and (e) not CL-compounds which turns into chemiluminescent ones after irradiation.

A few compounds belong to (a) and (b) groups. Only amino acids such as asparagine, arginine, isoleucine, threonine, serine, glutamic acid, proline, alanine, aspartic acid, glycine, valine and glutamine were indifferent to photoirradiation with low-pressure mercury lamps. Due to the low values observed for inositol and moroxidine (close to the base line deviation), these two drugs can be also included in group (a). The signal intensity decreases in the case of metamizol, morphine, streptomycin, dipyrone, captopril, perphenazine and ascorbic acid were the only compounds in which CL signal decreased. The diminution was specially important in basic media. This behaviour suggests the possibility of using low-pressure mercury lamps for removing potential interfering compounds such as ascorbic acid, widely used as excipient in pharmaceutical formulations (at pH 10 ascorbic acid was completely photodegraded).

For 36 (38%) of compounds a discreet increase of chemiluminescence (<10-folds) was observed. However, this group includes sulphamethoxypyridazina (×9.7), thioridazine (×9.7), chloropromazine (×8.3), citric acid (×8.9) and novocaine (×6.5), which present an increase of the I(ON)/I(OFF) ratio >5. Tryptophan, trimethoprim, isoniazid, acriflavinium chloride, phenothiazines, paracetamol, phenylephrine, epinephrine, amoxicillin, salicylic amide and tannic acid have ratios <10, but the absolute increases were of several hundreds of counts.

From an analytical point of view the most interesting Ph-CL effects are included in groups (d) and (e) and they are suitable for developing new and sensitive chemiluminescent analytical procedures.

The chemiluminometric response of 36 pharmaceuticals was increased by a factor ≥10. Twelve of them gave a I(ON)/I(OFF) >25, e.g. tyrosine (45.8), methionine (69), hydrochlorothiazide (10233), pyridoxine (39.5), Vitamin B1 (246), naphazoline (89.7), indomethacin (40.8), quinine (31.6), diphenhydramine (37.5), ampicillin (77.8), levamisole (25.2) and saccharin (28.2). The enhancing influence of photoirradiation on the CL behaviour can be also observed in the number of compounds which turned into strong chemiluminescent ones (CL intensity >1000 counts). It can be seen by an increased analytical signal for lysine (2617 counts), tyrosine (1336), nicotinamide (1699), pyridoxine (1416), naphazoline (1064), codeine (1105), ergotamine (1563), imipramine (1874), thioridazine (1917), chloropromazine (2880), norephedrine (1262), norepinephrine (3373), ampicillin (2019), doxycycline (2553), tetracycline (1328), tannic acid (1677), diethylestilbestrol (1280) and L-dopa (2767); an increase of several hundreds for cysteine (4022) and emetine (8152), and more than 10,000 for hydrochlorthiazide (20,464) and thiamine (44,000).

About 20% of drugs (group (e)) which did not present chemiluminescence or very weak chemiluminescence behaviour

Table 2
Ph-CL of monocyclic and bicyclic N-containing heterocyclics

Drug	H_2SO_4 0.001 M OFF/ON	H_2O OFF/ON	pH 4.5 OFF/ON	pH 7.1 ON/OFF	pH 8.6 OFF/ON	pH 9 OFF/ON	pH 10 OFF/ON	NaOH 0.001 M OFF/ON	NaOH 0.5 OFF/ON
Monocyclic N-containing heterocyclics									
Metamizol (pyrazole derivative)	1066/−826	1309/−943	1528/−1301	1513/−1271	1397/−1160	1334/−926	887/−653	1352/−1032	816/−148
Naphazoline (imidazole derivative)	8/+211	9/+148	8/+74	10/+74	8/+86	11/+62	10/+86	9/+98	8/+1064
Clotrimazole (imidazole derivative)	–/+265	–/+273	–/+264	–/+286	–/+241	–/+264	–/+196	–/+247	–/+119
Isoniazid (pyridine derivative)	206/+24	159/+54	151/+48	159/+40	130/+80	172/+88	159/+85	173/+146	206/+318
Pyridoxine (pyridine derivative)	–/+449	–/+584	–/+503	–/+629	–/+539	–/+674	14/+539	45/+472	67/+1416
Nicotinamide (pyridine derivative)	11/+131	6/+33	8/+107	10/+45	5/+1699	8/+23	10/+13	8/+26	17/+8
Trimethoprim (pyrimidine derivative)	2813/+333	2864/+469	2813/+229	2708/+521	2813/+312	2760/+183	2844/+125	3021/+312	2313/+135
Thiamine (pyrimidine derivative)	–/+44000	–/+19833	–/+7000	–/+2000	–/+4500	40/+9800	40/+4700	34/+4000	200/+8800
Bicyclic N-containing heterocyclics									
Caffeine (xanthine derivative)	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	119/+181
Theophylline (xanthine derivative)	–/+30	–/<20	–/<20	–/<20	–<20	–/<20	–/<20	–/<20	20/+87
Oxine (quinoline derivative)	862/−17	857/+16	764/−9	773/−10	828/−132	826/−247	830/−171	824/−165	1001/−344
Morphine(isoquinoline derivative)	435/−210	448/−12	455/−147	457/−157	466/−336	473/−344	447/−279	454/−393	447/−345
Dextromethorphan (isoquinoline derivative)	–/+622	–/+511	–/+178	–/+89	–/+76	–/+138	–/+142	–/+89	–/+1212
Codeine (isoquinoline derivative)	521/−20	625/−83	479/+125	520/+104	450/+216	675/+138	616/+188	579/+46	770/+1105
Emetine (isoquinoline derivative)	–/+652	–/+543	43/+1479	261/+5391	152/+3761	239/+5848	217/+5435	217/+5000	870/+8152
Indomethacin (indole derivative)	113/+256	132/+451	131/+321	141/+319	163/+140	145/+244	138/+398	146/+471	89/+3528
Ergotamine (indole derivative)	1902/+1077	2342/+493	2265/+40	2059/+265	2433/−564	1807/+642	2360/+973	1862/+1572	6324/+1563
Quinine (aminohydroxyalquilated quinolone)	847/+6918	360/+9875	988/+8188	212/+6484	353/+5153	282/+5224	988/+6777	494/+6565	8470/+212700
Riboflavin (pteridine derivative)	–/+641	–/+385	–/+513	–/+510	–/+833	–/+410	–/+359	–/+410	103/+1230

Table 3
Ph-CL of bicyclic N-S, tricyclic N and N-S heterocyclics and amines

Drug	H_2SO_4 0.001 M OFF/ON	H_2O OFF/ON	pH 4.5 OFF/ON	pH 7.1 ON/OFF	pH 8.6 OFF/ON	pH 9 OFF/ON	pH 10 OFF/ON	NaOH 0.001 M OFF/ON	NaOH 0.5 OFF/ON
Bicyclic N-S containing heterocyclics									
Hydrochlorothiazide	39/+207	7/+1630	12/+1556	10/+1364	5/+2342	5/+1621	5/+1208	5/+2299	5/+20464
Tricyclic N-containing heterocyclics									
Acridine	23/+16	21/+154	25/+102	21/+165	29/+280	24/+128	21/+88	26/+128	97/+379
Proflavin	73/+52	107/+17	98/+18	99/+29	87/+18	110/+15	124/+52	115/+29	109/+8
Imipramine	51/+255	76/+77	51/+77	51/+102	102/+281	153/+204	82/+153	92/+87	128/+1874
Acriflavinium chloride	244/+249	232/+82	221/+46	232/+82	221/+63	267/+203	260/+221	237/+65	302/+442
Tricyclic N-S containing heterocyclics									
Fluphenazine	885/+131	833/+625	885/+1198	1042/+963	833/+782	729/+130	781/−364	990/−156	1406/−416
Thioridazine	233/+833	194/+1694	200/+583	305/+1083	305/+1000	277/+1294	277/+1280	388/+1054	333/+1917
Chloropromazine	1967/+109	217/+272	217/+217	271/+130	326/+185	336/+2283	336/+2446	217/+326	543/+2880
Perphenazine	455/−206	465/−235	636/−404	550/−271	488/−229	481/−229	470/−199	453/−218	487/−220
Amines									
Amitryptiline (aliphatic amine)	45/+198	47/+198	21/+204	14/+210	16/+346	16/+290	27/+185	29/+168	3784/−737
Diphenhydramine (aliphatic amine)	17/+176	15/+188	22/+167	18/+343	13/+357	19/+684	15/+476	29/+314	339/+496
Streptomycin (aminoglycoside)	129/−100	143/−96	151/−103	189/−101	220/−120	222/−127	229/−126	219/−127	1195/+175
Paracetamol (aromatic amine)	102/+218	109/+239	108/+234	110/+254	121/+257	120/+267	98/+244	120/+230	147/+574
Phenylephrine (phenyl alkylamine)	249/+156	263/+150	279/+74	271/+20	267/+174	280/+121	276/+161	278/+169	837/+277
Lidocaine (aromatic amine)	11/+15	8/+4	7/+7	7/+7	39/+7	5/+15	5/+13	7/+13	7/+83
Nortryptiline (aliphatic amine)	7/+206	6/+151	5/+190	5/+163	5/+202	10/+147	20/+104	282/+33	310/+37
Ephedrine (phenyl alkylamine)	–/+539	–/+247	–/+195	–/200	–/+162	–/+180	–/+221	–/+266	–/+487
Norephedrine (phenyl alkylamine)	–/+91	18/+173	22/+144	–/+100	–/+111	22/+167	27/+111	–/+200	–/+1262
Epinephrine (phenyl alkyl amine)	339/+152	358/+95	335/+103	377/+297	347/+362	353/+750	377/+636	453/+495	63/+343
Norepinephrine (phenyl alkylamine)	127/+585	128/+835	128/+585	102/+508	108/+678	85/+1551	85/+1424	110/+500	195/+3373

Table 4
Ph-CL of nitrocompounds, carboxylic acids and polycarbocyclics

Drug	H_2SO_4 0.001 M OFF/ON	H_2O OFF/ON	pH 4.5 OFF/ON	pH 7.1 ON/OFF	pH 8.6 OFF/ON	pH 9 OFF/ON	pH 10 OFF/ON	NaOH 0.001 M OFF/ON	NaOH 0.5 OFF/ON
Nitrocompounds									
Chloramphenicol (aromatic nitrocompound)	–/+356	–/+436	–/+572	–/+758	–/+669	–/+581	–/+587	–/+633	–/+439
Metronidazole (heteroaromatic nitrocompound)	–/+37	24/+27	61/+112	6/+88	6/+71	24/+31	8/+61	10/+41	122/+265
Carboxylic acids									
Ampicillin (β-lactam derivative)	31/+1146	52/+2019	26/+1990	23/+1502	39/+1000	32/+1229	37/+1224	39/+1339	585/+1377
Amoxicillin (β-lactam derivative)	399/+12	383/+331	396/+1	403/+182	412/+161	407/+221	405/+150	413/+213	812/+602
Citric acid (aliphatic carboxylix acid)	19/+50	13/+46	13/+95	10/+62	16/+85	12/+40	12/+23	12/+95	53/+83
4-Aminobenzoic acid (aromatic craboxylic acid)	67/+714	91/+818	66/+937	73/+789	83/+685	67/+688	63/+692	71/+733	114/+575
Salicylic acid (aromatic carboxylic acid)	128/+100	131/+133	112/+147	105/+122	105/+256	108/+257	277/−38	112/+114	130/+489
Levodopa (amino acid)	326/+276	456/+65	456/+65	472/+49	456/+32	358/+619	374/+359	342/+130	3256/+2767
Ascorbic acid (vinylgous carboxylic acid hemi-ester)	1247/−124	1295/−643	1227/−900	1193/−967	1291/−1090	1295/−1084	1159/−1160	1220/−1170	430/−285
Polycarbocyclics									
Doxycycline	70/+574	51/+670	96/+543	83/+926	76/+734	76/+798	70/+638	114/+1021	127/+2553
Tetracycline	97/+880	48/+448	74/+410	64/+1264	64/+992	97/+1094	65/+736	97/+976	145/+1328

(close to zero) in some of the tested media of photodegradation, were clearly chemiluminescent after UV-irradiation. It was the case of lysine, histidine, tyrosine, phenylalanine, pyridoxine, Vitamin B1, theophylline, dextromethorphan, emetine, riboflavin, ephedrine, norephedrine, chloramphenicol, metronidazole, inositol, piperazinamide, clotrimazole, saccharin and benzocain. The most remarkable results (increase in counts) were: ephedrine (539), saccharin (564), dextromethorphan (622), emetine (652), pyridoxine (674), chloramphenicol (758), riboflavin (833), piperazinamide (877), norephedrine (1262) and Vitamin B1 (44,000). Only ephedrine, chloramphenicol, piperazinamide and clotrimazole were not chemiluminescent with lamp OFF and clearly chemiluminescent with lamp ON in all media. Based on a search employing the Analytical Abstract database (1980–2004) and the key words chemiluminescence and the name of pharmaceuticals, no previous reported works related to the direct chemiluminescence behaviour of these drugs were published. Chloramphenicol [18] has been determined by photoinduced chemiluminescence. For ephedrine, bis(2,4,6-trichlorophenyl)oxalate [26], and a derivatization strategy with dansyl chloride, 4-fluoro-7-nitrobenzofurazan or naphthalene-2,3-dicarboxaldehyde (II) [27] was employed. The obtained results confirmed thus the effectiveness of Ph-CL on the search of new and strong chemiluminescent systems.

The same search in the Analytical Abstract database was performed for all tested compounds. The search yielded negative results for metamizol, naphazoline, lidocaine, phenylbarbituric acid, piramidon, diesthylestilbestrol and novocaine. Nevertheless, the positive response with lamp OFF or ON was confirmed by the screening.

3.4. Photoinduced chemiluminescence of structurally related compounds

The influence of the chemical structure on the Ph-CL behaviour of pharmaceuticals can be studied by comparing the analytical signal for structurally related compounds (e.g. amino acids, sulphonic acid amides, phenothiazines, polycarbocyclics, N and N-S containing heterocyclics). Specially interesting are the compounds that present mimimal structural differences, as it is the case of the following pairs: amitryptiline–nortriptyline, ephedrine–norephedrine, epinephrine–norepinephrine, sulphamerazine–sulphadimidine, nicotinamide–pyridoxine, caffeine–theophylline, morphine–codeine, acridine–proflavin, doxycycline–tetracycline, 4-aminobenzoic acid–salicylic acid, ampicillin–amoxicillin and benzocaine–novocaine. These drugs differ on a functional group bonded to the chemical basic structure of the molecule. This difference supposes a minimum change on the chemical structure in comparison with the nucleus of the molecule.

Amino acids are the unique group which includes a significative number of compounds without native and photoinduced CL behaviour (e.g. asparagine, arginine, isoleucine, threonine, serine, glutamic acid, proline, alanine, aspartic acid, glycine, valine and glutamine). Positive results were obtained for aromatic amino acids (tyrosine, phenylalanine, tryptophan and histidine). Histidine was CL at high pH due probably to the lost of aro-

Table 5
Ph-CL of miscellaneous compounds

Drug	H_2SO_4 0.001 M OFF/ON	H_2O OFF/ON	pH 4.5 OFF/ON	pH 7.1 ON/OFF	pH 8.6 OFF/ON	pH 9 OFF/ON	pH 10 OFF/ON	NaOH 0.001 M OFF/ON	NaOH 0.5 OFF/ON
Others									
Vitamin B12 (corrinoid group)	31/+38	46/+2	33/+10	30/+8	39/+3	33/+2	27/+13	37/+45	308/+208
Levamisole (imidazothiazole)	14/+237	11/+301	10/+320	10/+247	26/+225	11/+295	14/+236	6/+299	33/+800
Phenylbarbituric acid (barbituric acid)	21/+225	11/+218	8/+212	10/+263	34/+152	7/+222	5/+184	4/+194	14/+279
Pyramidon (pyran derivatives)	176+/29	265/+367	189/−15	206/+64	215/+123	209/−89	183/+291	200/+447	276/+343
Salicylamide (amide of aromatic carboxylic acid)	32/+78	23/+121	29/+126	43/+399	29/+409	29/+259	24/+229	25/+327	44/+944
Moroxydine (biguanide)	–/–	–/–	–/–	5/+5	16/+1	–/+7	5/+3	5/+3	16/+6
Chloramine T (sulphur containing compound)	11/+126	14/+117	45/+76	10/+85	493/−369	17/+95	5/+119	5/+85	5/+324
Tannic acid (polyphenol)	124/+3	118/+11	132/+15	118/+31	127/+48	112/+42	124/+206	132/131	608/1677
Inositol (polyhydroxylic compound)	–/–	–/–	–/–	–/–	27/+7	–/–	–/–	–/–	–/+24
Piperazinamide (amide-aliphatic amine)	–/+39	–/+12	–/+360	–/+16	–/+877	–/+18	–/+10	–/+24	–/+82
Diethylstilbestrol (hydroxylated hydrocarbon)	72/+372	58/+436	59/+403	92/+461	103/+395	79/+447	68/+419	64/+549	69/+1280
Saccharin (sulphonic acid derivative)	–/+41	–/+42	–/+56	–/+58	–/+564	–/+126	–/+43	–/+49	–/+516
Benzocaine (ester of aromatic carboxylic acid)	23/+168	25/+30	27/+56	41/+45	25/+43	68/+39	–/–84	–/–	–/–
Dopamine (polyphenolic-aliphatic amine)	336/+78	339/+39	339/+52	339/+52	339/+65	326/+423	365/+248	456/+78	626/+235
Novocaine (aromatic amino ester)	34/67	50/16	49/40	42/230	46/95	42/165	38/114	43/108	233/938
Dipyrone (pyrazolone)	1066/−826	1309/−942	1528/−1301	1513/−1271	1397/−1161	1338/−926	887/−653	1352/−1032	816/−148
Captopril (pyrrolidine derivative)	860024/−7828	151732/8873	165400/−17469	164278/−39671	120186/−44114	164950/−115866	200428/−196293	14036/−314	146042/−105303
Luminol (cyclic carbonil hydrazide)	428/−25	425/+33	420/−42	425/−39	399/−5	377/+144	345/+70	379/+127	3591492/−5194
DNPO (bis-dinitrophenyloxalate)	55/+192	43/+68	50/+32	37/+53	56/+37	41/+34	50/+13	35/+35	25/+47

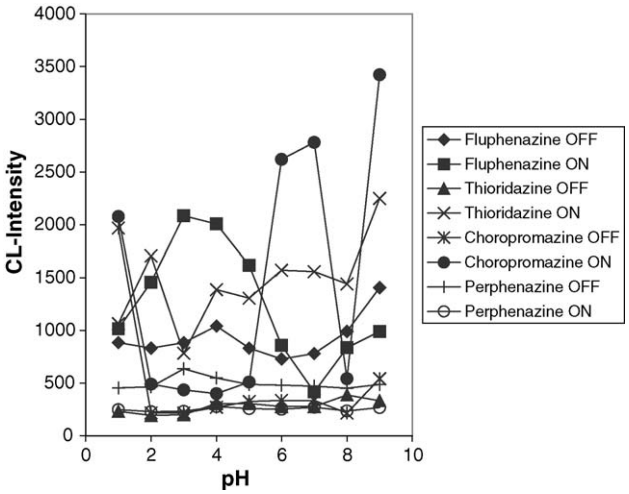

Fig. 2. Ph-CL of phenothiazines.

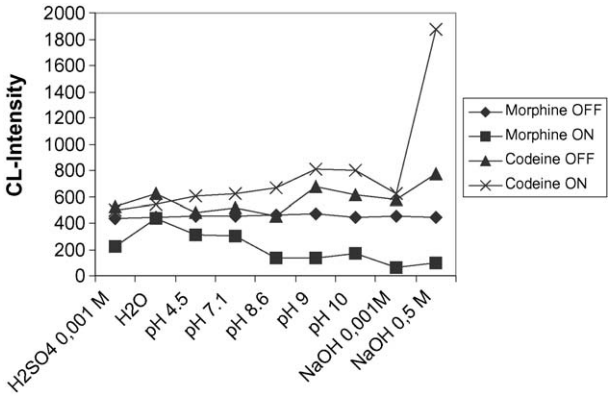

Fig. 3. Ph-CL of alkaloids.

maticity in acidic media caused by the protonation of the N of the heterocycle. Cysteine and methionine both containing easily oxidable groups presented the best results associated to experimental conditions in which the oxidation takes place in a faster way. Oxidation of cysteine is accelerated at higher pH, where the thiol is easily deprotonated; and methionine is oxidised by both chemical and photochemical pathways to form methionine sulphoxide in a further step into methionine sulphone. Leucine, isoleucine, valine, alanine and glycine present a hydrocarbon rest bonded to the carbon of the amino group; however, only leucine was clearly chemiluminescent after irradiation. This confirms the strong influence of little structural changes on the Ph-CL response.

Sulphonic acid amides can be derived from 4-amino-benzenesulphonic acid amide (NH_2-C_6H_4-SO_2-NHR). Although the nature of R group determines an important variety of structural types, the Ph-CL behaviour of sulphonic acid amides seems to be determined, on one hand by the sulphonamide group; and, on the other hand, by the amino group of the benzene nucleus. This was confirmed with the similar results obtained for the members of this family. All drugs were CL with lamp OFF and ON in acidic medium (the best results in M H_2SO_4 10^{-3} mol l^{-1}) and an enhancing effect of the CL was observed in all media after irradiation [19], being the analytical signals of the same order of magnitude for all sulphonamides in the same medium.

Phenothiazine is a linear condensed system of two benzene rings and a 4H-1,4-thiazine. Phenothiazine derivatives fluphenazine, thioridazine, chloropromazine and perphenazine were tested. Ph-CL properties seem to be influenced by the structure of the side chain at N10 and by the substitution of the benzene ring at the position 2. In spite of phenothiazine nucleus, there is not a regular trend on the behaviour for this family. Fluphenazine decreases the CL-emission in basic media, but thioridazine and chloropromazine are strong CL compounds in 0.5 mol l^{-1} NaOH with lamp ON. However, the native chemiluminescence of the perphenazine decreases in all media (see Fig. 2).

Monocyclic N-containing heterocyclics comprise an heterogeneous group of compounds: namely, pyrazole, imidazole, pyridine and pyrimidine derivatives. As in the case of phenothiazines the Ph-CL behaviour is not determined by the heterocyclic nucleus, but by the predominant structure of the bonded chains and groups. In fact, trimethoprim and thiamine are pyrimidine derivatives; nevertheless, trimethoprim is a strong CL emitter with lamp OFF, whereas thiamine is a non or weak CL compound and a very strong emitter after irradiation. A clear trend for the pyridine derivatives was not observed either. A similar comment can be applied to the heterogeneous group of the bycyclic N-containing heterocyclics (Table 2), amines (Table 3) and the heterogeneous group corresponding to Table 5.

Pharmaceuticals differing only in a methyl group such as the pairs amitryptiline–nortriptyline, ephedrine–norephedrine, epinephrine–norepinephrine, sulphamerazine–sulphadimidine and caffeine–theophylline, showed similar trends over the pH range except for 0.5 mol l^{-1} NaOH in which the non-methylated form exhibited the highest CL-emission.

Nevertheless, the change of a functional group with nitrogen or oxygen atoms bonded to the structural nucleus dramatically influenced the Ph-CL response. Morphine–codeine present morphinan as the parent structure. These two alkaloids only differ in the methoxy or hydroxy group attached to the C3 of the aromatic ring. This difference determines a diminution of the CL-emission of morphine after irradiation; and, on the other hand, an increase of the Ph-CL for codeine (see Fig. 3).

OH bonded to the benzene ring can be considered responsible of the weak emission of amoxicillin and the strong Ph-CL of ampicillin. This functional group supposes only a 5% of the molecular weight of the β-lactame derivative, remaining the rest of the complex chemical structure (see Fig. 4).

The presence or absence of amino groups attached to aromatic rings in acridine–proflavin and 4-aminobenzoic acid–salicylic acid; and the OH group in C4 and C6 of the six-member, annelated rings structure of the doxycycline–tetracycline couple determine significant differences against Ph-CL.

In general, taking into account the molecules of the groups (d) and (e), a molecule that experiments Ph-CL must have oxidable groups, aromaticity, weak bonded functional groups, heterocyclics and other ring-structures which can break down and

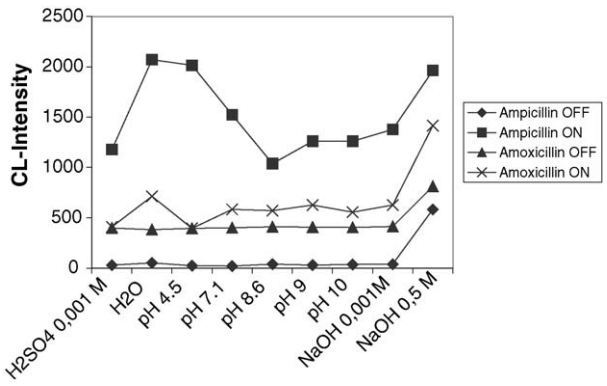

Fig. 4. Ph-CL of β-lactam derivative.

also give rise to dramatic structural changes. In this sense, the structural complexity is a factor that plays favourably, since it multiplies the possibilities of serious structural changes during the phototoirradiation step. Nevertheless, the presence of these structures is not a guarantee of a good Ph-CL behaviour.

4. Conclusions

Although it is relatively easy to find chemiluminescent (CL) molecules working on the field of direct liquid phase (especially employing strong oxidants like potassium permanganate), the molecules found as chemiluminescent are normally very weak CL compounds for obtaining suitable analytical CL-procedures. Therefore, it is mandatory to develop new strategies to enhance in a simple way the native chemiluminescence of such a compounds, and even to increase the number of compounds to be determined by direct chemiluminescence. The use of low-pressure mercury lamps in photodegradation processes allows the determination of compounds which do not present "native" chemiluminescence by using the appropriated medium of photodegradation (e.g. lysine, phenylalanine, chloramphenicol, saccharine, theophylline and emetine).

Moreover, the proposed systems led to strong chemiluminescence for chloramphenicol, piperazinamide, chlortrimazole, saccharine, thiamine and ephedrine. In general, a great improvement of the sensitivity was obtained. This is a very important factor in producing strong chemiluminescence systems for drug detection.

Photodegradation with low-pressure mercury lamps and chemiluminescent detection has a strong dependence with the used photodegradation medium. In general, a relevant increase of the intensity of chemiluminescent emission by photoirradiation was observed, mainly in strong alkaline medium (NaOH 0.5 mol l^{-1}). This suggests the possibility of a radical mechanism probably involving $^{\bullet}$OH radicals. However, from the structural differences between the drugs studied it must be assumed that more than one type of photochemical reaction is responsible for the chemiluminescent properties changes.

Ph-CL of pharmaceuticals is strongly dependent on the chemical structure. There is not a clear relation between chemical structure and chemiluminometric response after UV-irradiation. Structurally related compounds presenting scarcely differences in their structure show very often different chemiluminometric behaviour. This is the case of amitriptyline–nortriptyline, epineprrine–norepinephrine, ephedrine–norephedrine, sulphanerazine–sulphadimidine and caffeine–theophylline, which differ in a methyl group. From the family of amino acids leucine, isoleucine, valine, alanine and glycine present a hydrocarbon rest bonded to the carbon of the amino group; however, only leucine is a clearly chemiluminescent compound after irradiation. Similar comments can be applied to members of the phenothiazides family (chloropromazine, thioridazine and fluphenazine), morphine and codeine.

The results show the tandem photodegradation-CL detection as a promising approach for pharmaceutical analysis. Spectrum and power of the irradiation source determine strongly the mechanism of photodegradation and the photodegradation yields. Therefore, special regard must be paid to these aspects in further studies.

Although the short time of photoirradiation (circa 45 s), dramatic differences were observed for many pharmaceuticals.

The study of low-pressure mercury lamps as an effective means for pre-treatment of samples will extend this strategy to the determination of pharmaceuticals in biologically important samples, like foods, beverages by flow injection methods with chemiluminescence detection. Although only selected number of drugs have been described in this study, similar photochemical effects are expected for a much larger number of substances. Moreover, the study will also extend to analytes of environmental interest such as pesticides.

Because of the simplicity in the use of on-line irradiation and because of the fact that near 100% of the tested drugs exhibit chemiluminescence after irradiation with low-pressure mercury lamps, on-line photoirradiation and chemiluminescence detection can be found a wide application in HPLC post-column reaction.

Finally, because light acts as a "reagent" which has to be "added" on-line for photochemical reactions, many properties of an ideal system are exhibited, namely no excess of "reagent" reaches the detector.

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