

Chemiluminescence Methods (Present and Future)

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RESUMEN

Métodos de quimiluminiscencia (presente y futuro).

Este artículo da una visión general de los métodos de quimiluminiscencia en algunas de las aplicaciones más recientes en análisis de drogas, análisis del agua marina o la actividad antioxidante de productos naturales y de síntesis (incluyendo el aceite de oliva). Las consideraciones prácticas no están incluidas ya que el principal interés es establecer, a través de las aplicaciones mencionadas, que la quimiluminiscencia ha sido, es y será en los próximos años una herramienta versátil de la Química Analítica.

PALABRAS-CLAVE: *Actividad antioxidante - Análisis de agua marina - Análisis de drogas - Quimiluminiscencia.*

SUMMARY

Chemiluminescence methods (present and future).

This article provides a general review of chemiluminescent methods in some of their recent applications in drug analysis, sea water analysis or antioxidant activity of natural and synthetic products (including olive oil). Practical considerations are not included in the review since the main interest is to state, through the aforementioned applications, that chemiluminescence has been, is, and will be a versatile tool for Analytical Chemistry in future years.

KEY-WORDS: *Antioxidant activity - Chemiluminescence - Drug analysis - Radical scavenging - Seawater analysis.*

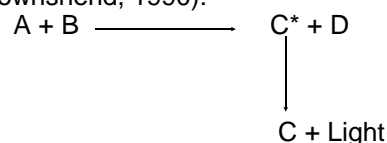
1. INTRODUCTION

We can define the term chemiluminescence (CL) as the emission of ultraviolet, visible or infra-red radiation from a molecule or atom as the result of the transition of an electronically excited state, having been produced as a consequence of a chemical reaction. This chemical reaction produces energy in sufficient amount to induce the transition of an

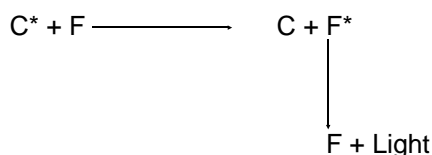
electron from its ground state to an excited electronic state. This electronic transition is often accompanied by vibrational and rotational changes in the molecule. In organic molecules, transitions from a π bonding to a π^* anti-bonding orbital ($\pi \rightarrow \pi^*$) or from a non-bonding to an anti-bonding orbital ($n \rightarrow \pi^*$) are most frequently encountered. (Dodeigne et al., 2000). When the reaction occurs in a living system or it is derived from one, the process is called bioluminescence (BL).

Luminescent reactions has been observed since ancient time, luminous animals are known in the Greek civilisation, however the first report of artificial chemiluminescence occurred in 1669. The German physician Henning Brand isolated from urine a substance that glowed continuously in the dark. He called the substance "phosphorus mirabilis", and it is better known today as white phosphorus (Barnett and Lewis, 1996). In the 19th century it was found that rather simple organic compounds could also give rise to chemiluminescence. Radziszewski observed the green light emission when oxygen was bubbled into an alkaline ethanolic solution of 2,4,5-triphenylimidazole (lophine) (Isacsson and Wettermark, 1974). This discovery was published in the year 1877. The term chemiluminescence was first coined in 1888 by Eilhardt Weidemann, as a part of his classification of "cold light" (luminescence). Forty years later, Albrecht in 1928 reported the luminescent properties of 5-amino-2,3-dihydrophthalazine-1,4-dione (luminol). Early research on CL was mainly focused on the observation of a reaction and investigation of the mechanism and the analytical applications of the phenomenon appeared in the literature in 1960s (Palilis and Calokerinos, 2000).

Nowadays, a lot of inorganic and organic CL reaction are known. A typical CL reaction would be (Townshend, 1990):



where (*) indicates an electronically excited state. Sometimes, The excited product (C^*) is an ineffective emitter, but it can transfer the excitation energy to an efficient fluorophore (F) added to the system:



Now, the emission is identical with the fluorescence of F and we can classify that as indirect, sensitised, or energy transfer chemiluminescence.

The light emission generated from a chemical reaction requires no light source for excitation, the analytical signal appears out of an essentially black background, and the only background signal is that of the photomultiplier tube's dark current. Analytically, the CL reactions are attractive due to: (a) Excellent sensibility and excellent detection limits because there is absence of source noise and scatter. (b) Sometimes high selectivity due to the limited number of available reactions. (c) Simple, robust and inexpensive instrumentation (Schmidt, 1999) suitable to both batch and flow analytical techniques. Furthermore, the introduction of flow injection analysis has made CL methods even more attractive because it is possible to mix sample and reagent rapidly with high reproducibility.

Chemiluminogenic reactions mainly occur in solution and in the gas phase. The most common or well known solution phase systems involve luminol (or its derivatives), oxalate esters, lucigenin (N,N'-dimethyl-9-9'-diacridinium nitrate) or its derivatives, ruthenium tris-bipyridine and luciferin. Gas phase examples include the ozone- and fluorine- induced, sodium vapor, and chlorine dioxide chemiluminescence detectors for gas chromatography (Van Fleet-Stalder and Chasteen).

The CL techniques have been applied to a great variety of analytes and samples. In literature we can

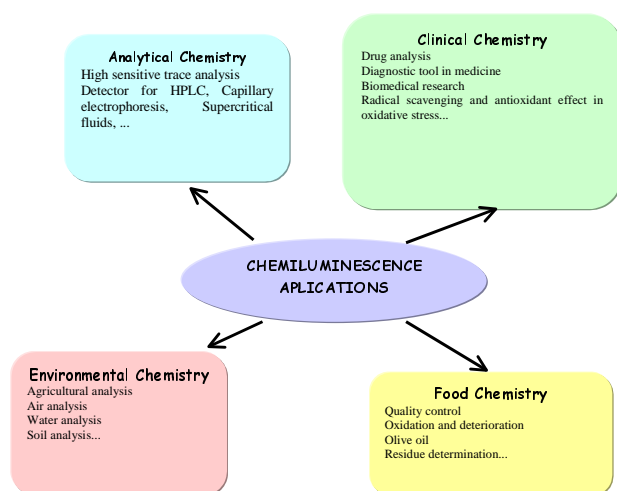


Figure 1
Some possible applications of CL methods.

find several dedicated reviews which show the importance of this techniques in some interesting fields as clinical (Kricka, 1994, Roda et al., 2000), food (Navas and Jiménez, 1996), environmental (Jiménez and Navas, 1997; Navas et al., 1997) or analytical chemistry (Huang and Fang, 2000; Jiménez and Navas, 1999; Palilis and Calokerinos, 2000). In the figure 1 we have summarised the most important applications of CL methods in several fields. Currently, it is possible to consider the CL methods as a powerful tools in Analytical Chemistry.

Today, the great variety of CL applications that have appeared in literature makes an exhaustive revision of them very difficult. In this review we have selected and summarised some interesting applications of CL techniques such as drug analysis, sea water analysis or antioxidant activity of natural and synthetic products, with the aim of giving an overview of the possibilities of these methods in some aspects of the environmental, clinical and food fields as olive oil. The selected bibliography is a small sample of the numerous reports appeared in the recent years since we have only tried to reflect the current tendency towards the CL techniques in these fields. Previously, the reagents and reactions which are the basis of the CL methods applied in the most described cases are briefly summarised.

2. CHEMILUMINESCENCE REACTIONS

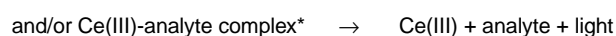
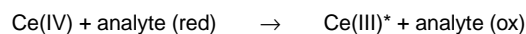
2.1. Chemiluminescence reaction with luminol

In aqueous solutions, the most commonly used chemiluminescent species is luminol. The chemiluminescence of luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) was first described by Albrecht in 1928. This compound reacts with a potent oxidizing agent (e.g. H_2O_2) in the presence of a catalyst (generally a metal or metal-containing compound or an enzyme) in alkaline solution to yield 3-aminophthalate in an excited electronic state which returns to ground state with the production of light.

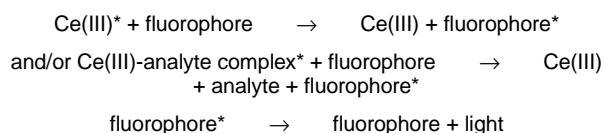
The most obvious use of the reaction has been to determine oxidants or compounds which interact with oxidant, but it is possible to determine metal ions by their catalysis of the CL reaction or products of enzyme-catalyzed reactions.

2.2. Chemiluminescence reaction with Ce(IV)

According to the investigation of CL properties of the fluorophore-sensitized Ce(IV) reaction system by Zhang et al. (1995), a possible CL mechanism of the reaction may be attributed to the following reactions:

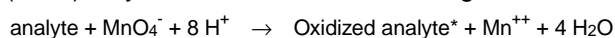


In the presence of a fluorophore, Ce(III) ions or the Ce(III)-analyte complex transfer the excess energy to the fluorophore which in turn generates CL emission:

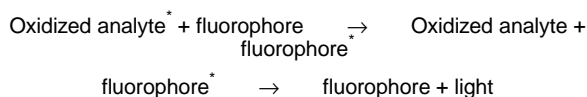


2.3. Chemiluminescence reaction with KMnO_4

A possible CL mechanism proposed by Aly et al. (1998) may be attributed to the following reactions:

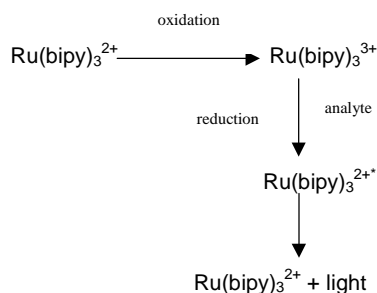


In the presence of a fluorophore, the energy resulting from the redox reaction can be effectively transferred to quinine which in turn generates CL emission.



2.4. Chemiluminescence reaction with tris (2,2'-bipyridyl)ruthenium (III)

Since the initial discovery of $\text{Ru}(\text{bipy})_3^{2+}$ chemiluminescence, its utility has been applied to the production of reactive oxidant, $\text{Ru}(\text{bipy})_3^{3+}$, followed by reduction, by an analyte species, to produce an emission of light (Aly et al., 2000 b):



Gerardi et al., 1999 summarises some mechanistic considerations of the CL reaction proposed by some authors.

3. ANTIOXIDANT ACTIVITY AND RADICAL SCAVENGING

The interest in scientific literature towards products which exhibiting a protective function in the oxidative changes which take place in foods (mainly, in relation to the production of reactive oxygen species) has been increasing in recent years, as reflected in the numerous reviews focussing on this topic (Robards and Antolovich, 1997, Robards et al., 1999, Prior and Guohua, 2000). Oxidative changes

in foods are important in terms of nutritional quality, flavor, odor, spoilage, and potential toxicity resulting from ingestion of oxidation reaction products (Yasaei et al., 1996). The term antioxidants in food is applied to those compounds which act interrupting the chain of free radicals (primary antioxidants), consuming oxygen or decomposing hydroperoxides into stable products (secondary antioxidants), (Kochlahr and Rosell cited by Galeano Diaz, 1996), or anyway act as enzymatic antioxidants or chelating agent.

On the other hand, oxidative stress is increasingly becoming an important hypothesis to explain the genesis of several pathologies, including cancer, atherosclerosis, aging (Blache et al. 1999) or Alzheimer's disease (McIntosh et al. 1997, Smith et al. 2000). Dietary factors (among others) are known to be involved in the production of reactive oxygen species which play important roles in oxidative stress, but on the other hand, protective functions of several components of foods and natural products (Phenolic compounds, vitamins...), in the aforementioned pathologies, seem to be largely due to their ability for scavenging reactive oxygen species.

In living systems, dietary antioxidants (α -tocopherol, β -carotene, ascorbic acid) and endogenous enzymes protect against oxidative damage (Moure et al., 2001). Enzymes such as superoxide dismutase (catalyze the dismutation of the superoxide radical to H_2O_2 and molecular oxygen) or catalase (catalyzes the breakdown of toxic H_2O_2 to water, preventing the secondary generation of hydroxyl radical) are involved in these defensive processes. Therefore, antioxidants are species which could play an important role in the protection from damage produced by oxidative processes that take place in our body.

In this section we have tried to compile some of the most recent reports on the possibilities of chemiluminescence methods as a reliable tool for detection and evaluation of the antioxidative activity of a great deal of natural and synthetic products, which work by mechanisms mentioned above. The growing interest in studying the antioxidant properties of some components of natural products is directly related to their protective function in foods and in human health, therefore papers reviewed here are focused in both directions.

At the same time, the summarized literature shows how CL methods have been widely applied as sensitive assay for detection and measurement of reactive oxygen species involved in the oxidative processes.

Oxygen-derived free radicals such as singlet molecular oxygen (SMO) ($^1\text{O}_2$), superoxide anion radical (SAR) (O_2^-) and hydroxyl radical (HR) ($\cdot\text{OH}$) are a very high reactive species. The identification of radical species is not easy due to their short lifetimes and different chemical properties. Their direct

detection and evaluation is difficult, electron spin resonance techniques usually being applied (Koleva et al. 2000). Methods for detection or monitoring these radicals, or compounds which present scavenger effects over these radicals, include CL methods based on reactions such as luminol, pyrogallol or Cypridina luciferin.

Suzuki et al. (1998) report methods for detecting active oxygen species (singlet oxygen and superoxide anion) using chemiluminescence phenomena. For singlet oxygen detection, its light emission in the near-infrared (1268 nm) is utilized. The authors make an application for measuring reaction rate constant of some chemiluminescent compounds and superoxide dismutase with $^1\text{O}_2$. The rate constants were measured by quenching the 1268 nm emission. They have also developed a method for measuring reaction rate constant of antioxidants with O_2^- , using quenching experiment of CL of Cypridina luciferin analogues and superoxide by superoxide dismutase. On the other hand, chemiluminescence from Cypridina luciferin has been used (Kruk et al., 2000) for determining the effects of thymoquinone (TQ), thymol (TOH) and dithymoquinone (TQ₂) [constituents of the volatile oil of black seed (*Nigella Sativa Linn*)] on reactions generating reactive oxygen species. The effect of TOH, TQ and TQ₂ on a light emission arising from the enzymatic (xanthine-oxidase-hypoxanthine-bovine albumin) and nonenzymatic system for generating O_2^- were followed by a chemiluminescence method.

Luminol can emit luminescence when superoxide anion radical is present. This CL reaction can serve as useful detection method for radicals or antioxidant. Decrease of chemiluminescence intensity derived from luminol and superoxide anion radical, generated from the enzyme reaction of xanthine oxidase (XO) with hypoxanthine, has been investigated as a screening method for the determination of antioxidants in real samples. The LC-CL method (Ogawa et al., 1999) has been applied to extracts of green tea leaves (*Thea sinensis* L.). The most potent antioxidant in green tea extracts was (-)-epicatechin, detected in the extracts diluted 2000 times. The authors conclude that, though the antioxidant might inhibit the CL by inhibiting XO, it prevents oxidation of luminol, and quench excited aminophthalate, as well as scavenging superoxide radical anion.

CL methods based on luminol reaction (in presence and in the absence of SOD) have been employed (Kondo et al., 1999) to elucidate the scavenging mechanisms of specific catechins, (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG), on peroxy radicals. 2,2'-Azobis(2-aminopropane) hydrochloride (AAPH), which generates peroxy radicals by its reaction with oxygen is used as an initiator of lipid peroxidation in

the liposomal system and of radical oxidation in the aqueous system. In EGC and EGCG the addition of SOD produced a remarkably inhibitory effect on CL, which allows the authors to suggest that the pyrogallol structure in the B ring, generates superoxide during the action.

Dapkevicius et al., (1999) have employed the inhibition of luminol chemiluminescence for the on-line detection of both, natural and synthetic antioxidants (rosmarinic acid, carnosic acid, α -tocopherol, *tert*-butylhydroxitoluene, *tert*-butylhydroquinone, carvacrol and thymol), separated by HPLC. Detection limits ($\mu\text{g ml}^{-1}$) reported by the authors were in the range 0.05 to 7.10. In a recent publication (Dapkevicius et al., 2001) these authors make an evaluation, optimization and comparison of two techniques (the on-line inhibition of luminol CL and the on-line 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) bleaching assay) for the on-line detection of analytes, which exhibiting radical scavenging activity, in HPLC eluates. Plant extracts of *Thymus vulgaris* L. and standards of some antioxidative compounds were used for the purpose of the investigation. The authors conclude that both methods are applicable for on-line screening of samples for natural radical scavengers, elucidation of lipid peroxidation/antioxidation mechanisms and radical scavenging processes, in general. Nevertheless, the DPPH \cdot seems to be more robust.

The total reactive antioxidant potential (TRAP) of "Sangre de Drago", a red viscous latex obtained from the bark of *Croton lechleri*, has been measured by monitoring the intensity of luminol-enhanced chemiluminescence (Desmarchelier et al., 1997) using 2,2'-azo-bis(2-amidinopropane) as a peroxy radical source. Results obtained suggest to the authors that Sangre de Drago is highly effective in scavenging peroxy and hydroxyl radicals at high concentrations. Nevertheless, at lower concentrations it presented prooxidant activity. Antioxidant activity has been tested using the hydroperoxide initiated chemiluminescence assay in rat liver homogenates. The latex was effective in capturing peroxy and hydroxyl radicals and in preventing oxidative DNA damage in aqueous. A similar study has been carried out by these authors in aqueous and methanolic extracts of several trees used in as anti-inflammatory in northeastern Brazil (Desmarchelier et al., 1999).

The chemiluminescence arising from pyrogallol oxidation, is a phenomenon during which many different O_2 species are formed and each of these species contribute to different mechanisms to the overall phenomenon (Thanasoulis et al., 1999). This fact has been used to study the influence of compounds and enzymes that trap or scavenge certain oxygen species (NaN_3 for singlet oxygen, SOD for

superoxide anion, and catalase and peroxidase for H_2O_2) that are active in oxidation processes.

The competitive reaction rate of various antioxidants, [butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and *t*-butyl hydroquinone (TBHQ)] and lipids have been studied to elucidate the roles and effectiveness of antioxidant in the prevention of food oxidation by singlet oxygen (Yasaei et al. 1996). Methyl linoleate (as a model lipid) is oxidized by singlet oxygen generated by irradiating the solution with added rose bengal. The lipids and antioxidant oxidation products were analyzed by HPLC with specific detection of the hydroperoxides by post-column chemiluminescence and/or iodometric detection. The obtained results allow to the authors conclude that, more efficient singlet oxygen scavengers that can be added to foods may be required for protection against singlet oxygen oxidation of lipids.

The hydroxylation of non chemiluminescent phthalic hydrazide by hydroxyl radicals to give the strongly chemiluminescent 3-hydroxyphthalic hydrazide supplies the basis for the CL method employed by Backa et al. (1997) for detecting hydroxyl radicals. Selectivity, sensitivity of the method and the influence of reaction conditions are presented. The authors review the applications (fungal degradation of food, in the bleaching of pulp with ozone and oxygen) of the CL method in both, quantitative and qualitative analyses of hydroxyl radicals.

Yildiz and Demiryük (1998) have reported that the hydroxyl radical is generated from the reaction between ferrous iron and molecular oxygen, and this reaction induces luminol chemiluminescence. They have demonstrated that CL signal is significantly inhibited in a concentration-dependent manner by either superoxide dismutase (SOD) or catalase, suggesting that superoxide and H_2O_2 are simultaneously generated. At the same time, specific hydroxyl radical scavengers (mannitol and dimethyl sulfoxide) also produce attenuation of ferrous iron induced chemiluminescence. Antioxidants, urate, ascorbate and methionine produced significant inhibitions in this chemiluminescence.

Kalitchin et al (1997) have reported the antioxidant activity of four cholesteryl esters of cinnamic acid derivatives (cholesteryl 2''-hydroxycinnamate, cholesteryl 3''-hydroxy cinnamate, cholesteryl 4''-hydroxycinnamate and cholesteryl 3'',4''-hydroxycinnamate, as well as cholestanyl 2''-hydroxycinnamate) by chemiluminescence. Tetraline reaction medium is chosen for this purpose due to its well known chemiluminescence and rate constant in oxidation radical chain reaction. The oxidation is initiated by azo-bis-*iso*-butyrate nitrile. The authors reported that though all the cholesteryl ester were efficient radical acceptors, cholesteryl 3'',4''-hydroxycinnamate resulted particularly efficient radical scavenger. Furthermore,

the activity of the inhibitors studied was comparable with that of β -naphthol, resorcinol and hydroquinone, determined in tetraline at 353 K. In a later report (Kalitchin et al. 1998), these authors continued making comparative studies on the antioxidant properties of cholesteryl esters of substituted cinnamic acids by chemiluminescence, in order to evaluate the effect of the methoxy group on their antioxidant properties.

Enhanced chemiluminescence and photo-chemiluminescence ((luminol-p-indophenol photo-induced chemiluminescence of luminol, respectively) methods have been utilized (Amarowicz and Raab, 1997) for establishing the effectiveness of leguminous extracts (white bean, pea, lentil, everlasting pea, faba bean, and broad bean) as natural antioxidants. All extracts of leguminous seeds investigated exhibited antioxidative properties to greater or lesser degree.

Red wine is a rich source of flavonoid antioxidants. On the other hand, radical reactions are thought to play an important role during beer oxidation, and it has been demonstrated that free radicals are produced in beer and reduce quality. The determination of antioxidant capacity of wine and the deterioration of beer quality (due to oxidative changes) by chemiluminescent methods have been reviewed in a precedent paper by us (Navas and Jiménez, 1999).

A thorough and very interesting review about lipid chemiluminescence as a book chapter (Wheatley, 1999) summarizes the scope of chemiluminescent methods on this topic including sections dedicated to chemiluminescent antioxidant assays or lipid peroxidation detection by CL methods based on different CL reactions (Luminol, peroxalates...) and electrogenerated and ultraweak chemiluminescence.

4. CHEMILUMINESCENCE AND OLIVE OIL

Flavonoids and other plant-derived phenolic compounds are known to exert strong anti-oxidative activity. It is known that the plant seeds, which are the source of edible oils, including olive oil, are abundant in these antioxidant compounds. These can help to protect against degenerative disorders of the cardiovascular system.

Miyazawa et al. (1995) and Sugawara et al. (1999) have analysed oils including olive oil. Mono-, bis-, and tris- hydroperoxides of triacylglycerols formed during autoxidation and photosensitized oxidation of oils were determined by reversed-phase high-performance liquid chromatography in combination with chemiluminescence detection. Later, this investigation group (Sugawara et al., 1999) studied by the same technique the determination of triglyceride hydroperoxides in olive

oils. The authors concluded that the CL-HPLC method, specific for the detection of hydroperoxides, should prove useful in studies of triacylglycerol oxidation in vegetable oils.

The suppression of the oxidation of triglyceride and methylesters of olive oil by additives of natural phenolic acids at 100°C was studied by Kasaikina et

al. (1997). The rate constants of the interaction of these acids with peroxy radicals in the oxidation of cumene at 60°C were determined by chemiluminescence method. The authors reported that the caffeic acid is the most efficient lipid antioxidant, exceeding ionol and α -tocopherol.

Table I
Analyte determination by chemiluminescence in seawater

ANALYTE	DETERMINATION BY	OBSERVATIONS	CL REACTION	DETECTION LIMIT OR MDC*	REFERENCE
Cobalt (II)	FIA - CL	Column preconcentration using 8-quinolol immobilized on silica gel	Gallic acid-hydrogen peroxide system	0,62 ng L ⁻¹ (3S)	Hirata et al. (1996)
Sulfur-containing compounds	GC with open tubular columns-CL detection		Specific sulfur detector	0,1 ng L ⁻¹	Savuk et al. (1995)
Arsenic (V) Phosphorus (V)	FIA - CL	Prior separation by ion chromatography. Applied to analyses of a seaweed reference material CRM 9 Sargasso	Luminol in an NaOH medium. Acid solution of molybdate added of metavanadate ion	10 µg L ⁻¹ 1 mg L ⁻¹	Fujiwara et al. (1996)
Ozone	CL-ozone sensor	On line measurement of the vertical turbulent ozone flux	CL of an organic dye adsorbed on silica gel in the reaction with ozone	< 0,1 ppb	Güsten et al. (1997)
Hydrogen peroxide	FIA - CL	The authors study the photogeneration of H ₂ O ₂ in marine waters Intercomparison with fluorescence decay technique	Luminol-Co(II)-H ₂ O ₂ in 0,1 M sodium carbonate 10-methyl-9-(p-formylphenyl)-acridinium carboxylate trifluoromethanesulfonate-hydrogen peroxide	10,6 nM H ₂ O ₂ (signal-to-noise=3) 5 nM	Price et al. (1998) Cooper et al. (2000)
Copper complexation	FIA - CL	Without preconcentration and with minimal sample perturbation	Reaction of copper with 1,10-phenantroline-hydrogen peroxide	0,1 nM in undiluted seawater	Zamzow et al. (1998)
Manganese	FIA - CL	Iron species removed through a 8-quinolol chelate resin column	Luminol-H ₂ O ₂	0,029 nM (lower) 4 mM (upper)	Okamura et al. (1998)
Iron	FIA - CL	Prior preconcentration using 8-hydroxyquinoline On hydrophilic vinyl polymer using Toyopearl HW-40C resin	Fe-catalysed oxidation of luminol by hydrogen peroxide and tryethylene tetramine as sensitizer	0.021 nM (3S) (averaging)	Jong et al. (1998) Jong et al. (2000)
Total dissolved iron	FIA - CL	Prior reduction with sulfite and preconcentration on an 8-hydroxyquinoline chelating resin column	Oxidation of luminol catalized by Fe ions, emitting blue light (λ_{max} 440 nm)	40 pM (3S)	Bowie et al. (1998)
Iron (II) and Total iron	FIA - CL	preconcentration on an Amberlite XAD-4 resin functionalized with N-hydroxy ethylethylenediamine	Brilliant sulfoflavine and H ₂ O ₂ reagent sol.	Iron (II) 0,80 nmol L ⁻¹ in sea water samples using a concentration of 2 nmol ⁻¹ iron (II).	Hirata et al. (1999)
Cobalt (II)	FIA - CL	Prior preconcentration using 8-hydroxyquinoline immobilized in Toyopearl	Pyrogallol-hydrogen peroxide-sodium hydroxide	5 pM (3S)	Cannizzaro et al. (2000)
Iron (II+III)		100 µM Na ₂ SO ₃ as iron (III) reducing agent	Luminol-dissolved oxygen as the oxidant	40 pM (3S)	
Phosphorus		Sorption preconcentration of phosphorus as a yellow vanadomolybdophosphoric heteropoly acid (HPA)	Alkaline-luminol reaction and HPA as oxidant	0.02 µg of P L ⁻¹ (in the presence of surfactant) 0.1 µg of P L ⁻¹ (in the	Zui and Birks (2000)

Table II
CL applications in pharmaceutical analysis

ANALYTE	CL METHOD	OBSERVATION	DETECTION LIMITS	REFERENCE
Ascorbic acid	Inhibition of vit. C in the CL reaction between luminol and ferricyanide	Luminol and ferricyanide immobilized on an anion-exchange resin column	$5,5 \times 10^{-3} \mu\text{g ml}^{-1}$	Zhang and Qin., (1996)
Vitamin B ₁₂	Cathalytic effect of cobalt(II) on the CL reaction between luminol and hydrogen peroxide	Luminol immobilized electrostatically on an anion-exchange column, and hydrogen peroxide electrochemically generated	$3,5 \times 10^{-4} \text{mg l}^{-1}$	Qin et al. (1997)
Pyridoxine hydrochloride	Enhancing effect of analyte on the CL generated by the oxidation of luminol with hydrogen peroxide in aqueous potassium hydroxide and sodium oxalate		$6 \mu\text{g ml}^{-1}$	Alwarthan and Aly (1998)
Etamsylate	Quenching effect of the analyte in the luminol hypochlorite system	Electrogenerated unstable reagent in a flow injection setup	$6 \times 10^{-10} \text{g ml}^{-1}$	Zhang et al. (1998a)
catecholamines (dopamine, adrenaline and isoprenaline)	Inhibition of the intensity of the CL from the luminol-hypochlorite system	The hypochlorite was generated electrochemically on-line	Dopamine: $6 \times 10^{-10} \text{g ml}^{-1}$ Adrenaline: $8 \times 10^{-10} \text{g ml}^{-1}$ Isoprenaline: $8 \times 10^{-10} \text{g ml}^{-1}$	Zhang et al. (1998b)
Tannic acid	Inhibition of the CL of the luminol-H ₂ O ₂ -Cu ⁺⁺ system by tannic acid		$9 \times 10^{-9} \text{mol l}^{-1}$	Cui et al. (1998)
β -lactam antibiotics	CL was directly produced by the reaction of β -lactam antibiotics with luminol in the presence of a catalyst in an alkaline solution without H ₂ O ₂	hexacyanoferrate (III) and hexacyanoferrate (II) were used as catalyst	Between 2×10^{-1} to $20 \mu\text{g ml}^{-1}$	Kubo et al. (1999)
Isoniazid	Enhancement effect of analyte on the weak emission of light produced by CL reaction between BrO ⁻ and luminol	The BrO ⁻ was electrogenerated on-line in KBr solution	$7 \times 10^{-9} \text{g ml}^{-1}$	Zheng and Zhang (1999)
Lactate	Fe ²⁺ generated detected by the CL reaction of luminol system without added oxidant	Decomposition of lactate in the presence of UO ₂ ²⁺ and Fe ³⁺ , and production of Fe ²⁺	2ng ml^{-1}	Pérez-Ruiz et al. (1999a)
Vitamin K ₃	Monitorization of H ₂ O ₂ produced by CL reaction with luminol catalysed by hematin	Photooxidation of ethanol sensitized by vitamin K ₃ to yield H ₂ O ₂	$2,03 \times 10^{-9} \text{mol l}^{-1}$	Pérez-Ruiz et al. (1999b)
Chloramphenicol	CL detection of photolytic fragments whit luminol - Co(II) system	Photodegradation of nitro compounds	$3 \times 10^{-9} \text{mol l}^{-1}$	David et al. (2000)
Sulbactam sodium and clavulanic acid	Enhancing effect on the CL generated by the oxidation of luminol with H ₂ O ₂ in alkaline medium		Sulbactam sodium: $0,05 \mu\text{g ml}^{-1}$. Clavulanic acid: $0,01 \mu\text{g ml}^{-1}$	Aly et al. (2000a)
Atropine	Analytical CL signal produced by cetyltrimethylammonium chloride – luminol – ion pair complex of tetrachloroaurate (III) with atropinium	On line ion-pair formation and solvent extraction method copuled with reversed micellar mediated CL		Fujiwara et al. (2000)
Penicillamine	CL reaction of thiol containing drugs with Ce(IV) in H ₂ SO ₄ medium	Quinine as fluorescer	$15 \text{pmol } \mu\text{l}^{-1}$ as thiol	Zhang et al. (1996)
Tiopronin	Oxidation by Ce (IV) in dilute sulfuric medium	Sensitized by quinine	$0,34 \mu\text{M}$	Zhao et al. (1997)
Some phenothiazines	CL induced by the oxidation of drugs with Ce (IV) in acid medium	Rhodamine B as sensitizer	Fluphenazine hydrochloride: $0,01 \mu\text{g ml}^{-1}$ Levomopromazine hydrochloride and trimeprazine tartrate: $0,1 \mu\text{g ml}^{-1}$	Aly et al. (1998a)
Tiopronin	CL reaction of tiopronin with Ce (IV) in sulfuric acid medium	Rhodamine 6G and quinine as fluorophors	$3,6 \times 10^{-8} \text{M}$	Pérez-Ruiz et al. (1998)
Hydrochlorothiazide	CL reaction of hydrochlorothiazide with Ce (IV) in sulfuric acid	Sensitized by rhodamine 6G	$0,15 \mu\text{mol l}^{-1}$	Ouyang et al. (1998)
Analgin	CL reaction of analgin with Ce(IV) sulfate in sulfuric acid	Sensitized by Rhodamine 6G	$0,02 \text{mg ml}^{-1}$	Huang et al. (1999)
Hydrochlorothiazide and captopril	CL reaction with Ce (IV) in sulfuric acid medium	Sensitized by rhodamine 6G	Hydrochlorothiazide: $0,2 \mu\text{mol l}^{-1}$ Captopril: $2,7 \mu\text{mol l}^{-1}$	Ouyang et al. (1999)
Sodium 2-mercaptoethane sulfonate	CL reaction of thiol with Ce (IV) in sulfuric acid medium	Quinine as sensitizer	$1,38 \mu\text{g l}^{-1}$	Capitán-Vallvey et al. (2000)
Cefadroxil monohydrate	CL reaction of analyte with potassium permanganate in sulfuric acid	Sensitized by quinine	$0,05 \mu\text{g ml}^{-1}$	Aly et al. (1998b)
Naltrexone	CL reaction with potassium permanganate in sulfuric acid medium	without FIA	25ng ml^{-1}	Campigliio (1998)
Perphenazine	CL reaction with potassium permanganate in sulfuric acid medium	Method with flow injection		Sultan et al. (1999)
Salbutamol and ranitidine	CL reaction with acid potassium permanganate in sodium polyphosphate	Weak CL from the reaction between ranitidine and KMnO ₄	Salbutamol: $2,5 \times 10^{-8} \text{M}$ Ranitidine: $1 \times 10^{-5} \text{M}$	Barnett et al. (1999)
Salicylamide	Oxidation of the drug by potassium permanganate in dilute sulfuric acid	A FI method for salicylamide	30ng ml^{-1}	Fuster Mestre et al. (1999)
Procaine, benzocaine and tetracaine	Oxidation of the analytes by permanganate in sulfuric acid in the presence of various CL enhancers	Determination by sequential injection analysis with CL detection	Procaine hydrochloride: and benzocaine: $0,3 \mu\text{g ml}^{-1}$ Tetracaine hydrochloride: $0,1 \mu\text{g ml}^{-1}$	Paseková and Polásek (2000)
Thioridazine hydrochloride	Oxidation by potassium permanganate in acidic medium	Flow-injection analysis	$1,2 \times 10^{-6} \text{M}$	Kojlo et al. (2000)

Table II (Cont.)

Codeine	Ru(bpy) ₃ ²⁺ and codeine are oxidized and upon reacting generate the excited product Ru(bpy) ₃ ²⁺	Complex immobilized in a sintetic matrix	20µM	Michel et al. (1999a)
Codeine	Ruthenium complex (Ru(bpy) ₃ ²⁺ and Ru(bpy) ₂ (phen) ²⁺) and codeine are oxidized and upon reacting generate the excited product	Measurements carried out in batch and FIA mode	Ru(bpy) ₃ ²⁺ and batch mode: 0,5µM Ru(bpy) ₂ (phen) ²⁺ and batch mode: 0,1µM Ru(bpy) ₃ ²⁺ and FIA mode: 100µM Ru(bpy) ₂ (phen) ²⁺ and FIA mode: 50 µM	Michel et al. (1999b)
Tricyclic antidepressants	CL reaction between Ru(bpy) ₃ ²⁺ and the tertiary amino groups		Amitriptyline: 0,09 µg ml ⁻¹ Doxepin: 0 10 µg ml ⁻¹ Nortriptyline: 0,31 µg ml ⁻¹ Promazine: 0,16 µg ml ⁻¹ Chlorpromazine: 0,24 µg ml ⁻¹	Greenway and Dolman (1999)
Ranitidine	CL reaction between Ru(bpy) ₃ ²⁺ and a tertiary and two secondary amine groups	Flow injection analysis with CL detection	6 x 10 ⁻⁷ M	Barnett et al. (1999)
Tetracyclines	CL system of Ru(bpy) ₃ ²⁺ oxidized by acidic permanganate in presence of Mn(II)	The light emission intensity is enhanced when the analyte are also present in the reaction system	Tetracycline: 2,0 x 10 ⁻⁸ g ml ⁻¹ Chlortetracycline: 1,0 x 10 ⁻⁸ g ml ⁻¹ Oxytetracycline: 2,0 x 10 ⁻⁸ g ml ⁻¹	Han et al. (1999)
Codeine	Ru(bpy) ₃ ²⁺ CL reaction	Miniature electrochemiluminescence detector	100µM	L'Hostis et al. (2000)
Flufenamic and mefenamic acids	Ru(bpy) ₃ ²⁺ CL reaction	Chemical generation of Ru(bpy) ₃ ²⁺ by mixing two streams containing solution of Ru(bpy) ₃ ²⁺ and acid Ce (IV)	Flufenamic acid: 3,6 x 10 ⁻⁹ M Mefenamic acid: 2,1 x 10 ⁻⁷ M	Aly et al. (2000b)
Fluoroquinolone derivatives	CL reaction of analyte with Ru(bpy) ₃ ²⁺ and Ce(IV) in sulfuric acid medium	Ce(IV) used to produce Ru(bpy) ₃ ²⁺ wich is used for determination of analyte	Norfloxacin: 2 6 x 10 ⁻⁸ M Ciprofloxacin: 2,6 x 10 ⁻⁸ M Ofloxacin: 5,5 x 10 ⁻⁹ M	Aly et al., (2001)

De la Puerta et al. (1999) have employed the CL to study the effects of polyphenolic compounds from virgin olive oils (tyrosol, hydroxytyrosol, oleuropein and caffeic acid) in the inhibition of leukocyte 5-lipoxygenase by the quenching effect produced on the CL signal due to reactive oxygen species generated by phorbol myristate acetate-stimulated rat leukocytes. This same investigation group (Gutierrez et al., 2001) have examined the effects of polyphenolic compounds from virgin olive oils on the non-enzymatic lipid peroxidation induced by ascorbate-Fe²⁺ of rat liver microsomes by chemiluminescence. The obtained result showed that the principal phenolics present within the polar fraction of Virgin olive oil possess an array of potentially beneficial lipoxygenase-inhibitory, prostaglandin-sparing, and antioxidant properties.

Sawa et al. (1998) and Kanazawa et al. (2000) have quantified the generation of lipid peroxy radicals (LOO[•]) from oxidized oils and edible oils (including Extra virgin and Virgin olive oils) by means of a luminol-enhanced chemiluminescence assay and their implication in human health. The authors concluded that a large proportion of anti-oxidant activity, as well as radical scavenging of edible oils can be lost by the conventional process of oil refining and they report as Extra virgin and Virgin olive oils are rich in LOO[•] Scavengers, so they are preferred not only for an anticarcinogenic potential, but also for prevention of reactive oxygen related diseases.

5. ANALYTE SEAWATER DETERMINATION BY CHEMILUMINESCENCE

Most of the water of the Earth's surface is in the ocean. Because of its chemical and physical properties, this water has had a great influence on the continuing biochemical evolution of this planet (Libes, 1992). Seawater constitutes a source of a wide variety of chemical ions considered as major constituents (Mg²⁺, Cl⁻, Na⁺, Ca²⁺, SO₄²⁻...), nevertheless, there are a great number of other elements or compounds which exist at trace levels and exhibit an important function in marine geochemistry, for instance, iron and manganese are known to be essential micronutrients for marine organisms. The analysis of ultra-trace elements in seawater samples is one of the most difficult analytical tasks in the field of environmental monitoring as extremely low detection limits for elements "buried" in a highly saline matrix are required (Ferradello et al. 2001).

One of the utilities of Chemiluminescent methods in Analytical Chemistry is the determination of trace metals due to the fact that a great deal of CL reactions require the presence of metal just as a catalyst or by its redox properties. The application of CL methods to seawater analysis is compiled in Table I.

6. CHEMILUMINESCENCE IN PHARMACEUTICAL SAMPLES

Accurate determination of drugs in pharmaceutical preparations is very important in the pharmaceutical industries. A variety of techniques have been used in the determination such as spectrophotometry (Travis et al., 1999; Sakiara et al., 1999, Altinoz and Dursun, 2000), spectrofluorimetry (Gatti et al., 2000, Rizk et al., 2000), electrochemical detection (Yun et al., 1999, Wang, 2000), etc. The analytical evaluation of commercially significant pharmaceutical drugs using chemiluminescence detection has attracted considerable attention in recent years, due to their higher sensitivity and their very simple instrumentation (no monochromator required). Moreover, the reproducibility and selectivity can be excellent by combination with a flow injection method.

Table II summarises the most important CL application to the determination of drugs in pharmaceutical preparation. The CL reaction of luminol can serve as a basis for the determination of analytes which can enhance (pyridoxine hydrochloride, isoniazid, sulbactam sodium or clavulanic acid) or inhibit (ascorbic acid, etamsylate, some catecholamines or tannic acid) the chemiluminescence. Furthermore, other analytes can be determined because they directly react with luminol (β -lactam antibiotics) or they produce some compound that would react with luminol (lactate or vitamin K₃).

Other interesting pharmaceutical analytes can be oxidised by Ce(IV) in sulfuric acid medium and this CL reaction can assist in their quantification. The reaction can be sensitised by several fluorophors, such as quinine (penicillamine, tiopronin, or sodium 2-mercaptoethane sulfonate) and rhodamine 6G (some phenothiazines, tiopronin, hydrochlorothiazide, analgin or captopril). In the same way, some drugs can be oxidised and determined by potassium permanganate in a sulfuric acid medium. Usually, the method implies a FIA procedure but Campliglio (1998) proposes the determination without FIA.

Numerous analytical applications of orange emission of tris(2,2'-bipyridyl)ruthenium(II) in acid solution have appeared in literature. In the table we have summarised some analytical applications in pharmaceutical preparation as determination of codeine, tricyclic antidepressants, ranitidine, tetracyclines, some fluoroquinolone derivatives or flufenamic and mefenamic acids.

7. CONCLUSIONS

With the increasing demand for highly sensitive and selective analyses in many areas of analytical sciences, chemiluminescence techniques continue to provide important and fascinating fields of research.

In this paper we have selected and reviewed the most recent literature in relation to three relevant fields of scientific, clinical, and environmental interest, in order to show how chemiluminescence is a living technique, with a plethora of real possibilities. Likewise, we have tried to show the role that the chemiluminescence plays in the determination of the antioxidant activity of natural products, with special mention to the olive oil.

Nowadays, technological advances happen very quickly, and this has enabled CL methods to improve and has led to an increase in its fields of applications. The awareness of new CL reactions, the coupling of these new reactions with others of analytical interest and, any case, the increase of new detection systems (replacement of the photon counting photomultiplier for the photodiode) have contributed to expanding the range of the applications for CL techniques. We will conclude by saying that CL is now and it will continue being a technique capable of solving analytical problems for the new millennium.

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