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RAPID COMMUNICATION

Review of chemiluminescent methods in food analysis

M. J. Navas & A. M. Jiménez

Department of Analytical Chemistry, Faculty of Pharmacy, University of Seville, Seville, Spain

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The purpose of the present review is to sketch out the scope of chemiluminescence in food analysis. Practical considerations are discussed. Specific applications to the determination of N-nitroso compounds, sugars, food oxidation, hormonal anabolics and metabolites, metals and other interesting compounds. There is also discussion on how alcohols, enzymes, etc. have been revised. Possibilities and limitations of the various reaction and detection systems are evaluated.

INTRODUCTION

Chemiluminescence can be defined 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. There are many inorganic and organic chemical reactions that are known to produce light. A typical reaction would be: $A + B \rightarrow C^* + D\,C^* \rightarrow C + \text{Light}$ where (*) indicates an excited state (Townshend, 1990). This would be a rather simple case and would be classified as direct chemiluminescence. Sometimes C* is an ineffective transmitter, but by excitement energy transfer to an effective fluorophore (F) added to the system, a considerable increase of luminescence intensity is obtained: $C^* + F \rightarrow C + F^*\ F^* \rightarrow F + \text{Light}$

This would be classified as indirect sensitized or energy transfer chemiluminescence.

Chemiluminescence in analytical chemistry has numerous advantages such as superior sensitivity, safety and controllable emission rate, but it has some disadvantages such as poor reproducibility and long observation times, although not all the reactions are slow. There are numerous reports published (Grayeski, 1987; Robards & Worsfold, 1992; Miller, 1984; De Jong & Kwakman, 1989) about the importance of chemiluminescence methods in analytical chemistry and their applications in the determination of a great variety of compounds. In this review chemiluminescent methods used in food analysis in the last 10 years are reported and discussed. In addition, the reagents and reactions which are the basis of these methods are briefly summarized.

REAGENTS AND REACTIONS IN CHEMILUMINESCENT METHODS

Luminol reaction

The chemiluminescence of luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) was first described by Albrecht in 1928 (Albrecht, 1928), and involves the oxidation of luminol in a basic solution (pH 10–11), generating an energy-rich intermediate with subsequent light emission of the aminophthalic acid. To obtain chemiluminescence from luminol in water, a potent oxidizing agent (e.g. H_2O_2) is needed. The reaction is catalyzed by metal ions such as Co(II), Cu(II) and Fe(II), etc., and by enzymes such as horseradish peroxidase, microperoxidase, etc.

The most obvious use of the reaction has been to determine oxidants, but it has also been used to determine compounds which interact with the oxidant. Determination of metal ions by their catalysis of the luminol reactions has been reported on numerous occasions. The effect of chelation of the catalytic metal ion has also been utilized. Polyamines such as ethylenediamine have been determined by reaction with salicylaldehyde to form a Schiff's base, which enhances the catalytic activity of manganese in the luminol–H_2O_2 reaction. This type of reaction has also been used to distinguish between chelated and non- or weakly-complexed chromium (III). An important use of luminol reaction is to detect the products of enzyme-catalyzed reactions. The luminol is used to detect H_2O_2 produced in such a reaction. Glucose, for example, could be determined by oxidation in the presence of glucose

oxidase to produce H_2O_2. Some compounds can be determined by a sequence of enzyme-catalyzed reactions culminating in H_2O_2. The luminol is also employed as a label in immunological methods. N-(4-Aminobutyl)-N-ethylisoluminol (ABEI) was used for the labelling of fatty acids, several amines and hormonal anabolics. This method should replace the more conventional radioimmunoassay method.

Lucigenin reaction

The oxidation of lucigenin (bis-N-methylacridinium nitrate) occurs in basic solution producing light emission from the excited state of N-methylacridone. The oxidation of lucigenin is achieved generally with a peroxide and it is catalyzed by a range of metal ions including Bi(III), which does not catalyse the luminol reaction. The chemiluminescence is also observed with a reductant and oxygen. Because oxidation and reduction steps are involved, it is analytically possible to measure both oxidants and reductants. Several analytes, oxidants such as hydrogen peroxide, some metals, and reductants such as ascorbic acid and fructose,have been determined.

Nitric oxide–ozone reaction

Nitrogen containing components can be determined using the chemiluminescent reaction of nitrogen oxide (Isacsson & Wettermark, 1974) with ozone: $NO + O_3 \rightarrow NO_2^* + O_2$ $NO_2^* \rightarrow NO_2 + \text{Light}$

Nitrates, nitrites and nitrosamines (Kricka & Thorpe, 1983) can be detected using a previous reduction to nitrogen oxide and a posterior reaction with ozone. Some important applications of this reaction in food analysis will be described in the next section.

APPLICATION OF CHEMILUMINESCENT METHODS

Detection of nitrogen containing components in foods

Chromatographic techniques, gas chromatography (GC) or high-performance liquid chromatography (HPLC), in combination with chemiluminescent detection (CLD), have been extensively applied to the identification and quantification of N-components in foods. The detection mechanism usually used is based on the nitric oxide reaction previously described. This process results in the formation of nitrogen dioxide in the excitated state (NO_2^*). Light emitted by this chemical reaction is detected. Recently, a post-column chemiluminescence detector using bis(2-nitrophenyl) oxalate and hydrogen peroxide as chemiluminogenic reagents has been used (Fu *et al.*, 1993). Investigated compounds include a wide variety of them like amino acids, peptides and proteins, nitrogen-containing components in flavors and essential oils, nitrites and nitrates, and N-nitroso compounds either as a group or N-nitrosamines, in particular. Studies carried out during the past years in this topic have paid considerable attention to the analysis of food for the presence of N-nitroso compounds, so this section has been focused especially on those compounds.

N-nitroso compounds are potent carcinogens, often at very low concentrations. This fact and the occurrence of this compound in human food makes their detection of great interest. The complexity of the composition of food samples is such that the determination of each individual N-nitroso compound would be impossible for a large number of samples, therefore studies have been mainly directed to the examination of N-nitroso compounds as a group. N-nitrosamines are usually differentiated into volatile and non-volatile nitrosamines. While the first can be removed easily from a food matrix by distillation and separated by gas chromatography, the physical characteristics of non-volatile nitrosamines make them difficult to extract quantitatively, so development of suitable analytical methods has been much more difficult than for volatile nitrosamines. Since amines occur ubiquitously in practically all foods, and nitrosating agents also occur quite commonly, these compounds have also been studied as precursors of endogenous N-nitroso compounds in foodstuffs (Scalan & Reyes, 1985).

An extensive review in relation to chemiluminescence and N-nitosamines in foods has been related by Scalan (1984) as a book chapter. The sensitivity and selectivity of the chemiluminescent-based detection system, particularly Thermal Energy Analyzer (TEA) in combination with GC or HPLC are discussed. Advantages and limitations of other detectors used are compared. Approaches used to deal with artifactual formation of nitrosamines and to confirm the identity of nitrosamines are also presented.

All attempts to determine N-nitroso compounds as a group have been based on the ability to make a complete breakdown of the bond between the nitrosyl group and the amino part of the molecule. N-nitroso compounds have been determined successfully as a group through the nitric oxide radical generated by different procedures (Denitrosation, Pyrolysis, Photolytic mode, etc.) and a chemiluminescent detector to measure the infra-red emission resulting from the interaction of this gas with ozone.

Walters *et al.* (1978) describe an analytical system based on a chemiluminescence detector for determination of total N-nitroso compounds. The sample is denitrosated according to Eisebrand and Preussman (cited by these authors) with acetic acid in refluxing ethylacetate to release nitric oxide from potentially interfering species such as labile compounds and inorganic nitrite. Nitric oxide is then selectively released from N-nitroso compounds by the denitrosation with hydrogen bromide. Conditions were chosen in order to obtain nitric oxide rather than nitrosil bromide. The latter is very reactive and can be completely lost by its interactions with components of foods. The method has been applied successfully to the determination of N-nitrosarcosine (a

non-volatile nitrosamine) on a food matrix. Pitfalls, artifacts and false positive responses related to the determination of N-nitroso compounds have been reported by several investigators.

Walters & Smith (1983) have underlined the importance of selectivity in the determination of N-nitroso compounds as a group trying to account for the evolution of nitric oxide from compound derived from nitrite, and pointing out the importance to ensure that evolution of nitrogen oxide from other compounds has ceased before the determination of N-nitrosamines and N-nitrosamides. In this way, the technique previously described has been applied to the determination of the apparent N-nitroso content of a range of food and beverages (Massey *et al.*, 1984b), using a thermal Energy Analyser. Foodstuffs (biscuit, dried milk, dried soups, dried coffee, tea, cocoa/chocolate, canned cured meats, bacon and beer) were selected on the basis that they either were known to contain volatile nitrosamines, or that they might have been subjected to nitrosating conditions during production or processing. The response associated with the hydrogen bromide reagent itself can be substantially reduced by treating the apparatus with an excess (10 ml) of the denitrosation reagent at the beginning of each day. Effects of high levels of nitrate and of the sample size on the effective detection limit were studied. Authors concluded total N-nitroso contents down to 10 μg (N–NO)/kg can be measured reproducible on a 1-g sample.

Application of this method to the trace analysis of aqueous and solid samples is reported by Massey *et al.* (1984*a*) with an investigation of the effects on the accuracy and sensitivity of the assay as applied to aqueous analytes. An equation is proposed in order to correct the detector response with respect to successive injections of the hydrogen bromide denitrosation reagent. Their effectiveness is further confirmed in beer samples and in the analysis of maturing whisky samples containing an unusually high concentration of N-nitrosodimethylamine (NDMA). Water effect on the accuracy and sensitivity of the total N-nitroso assay are studied. The magnitude and significance of the false-positive response from nitrate is discussed in relation to the analysis of cured meat.

Bouchikhi *et al.* (1989) describe some precautions to be taken in the analysis of total N-nitroso compounds to prevent some pitfalls in results. An apparatus-related response derived from reaction between hydrogen bromide and active sites (glass apparatus used) is also detected. The suggested recommendations allowed a good reproducibility of the results obtained of six determinations of 1.0-g aliquots of French cheese, by the same operator on the same day, using the some chemilumninescence analyser, (coefficient of variation = 4.3%).

Castegnaro *et al.* (1987) presented the first valuable results in a series of collaborative studies initiated by the Commission of Food Chemistry of the International Union of Pure and Applied Chemistry. One of the most important conclusions was the necessity to clean the apparatus very thoroughly between analysis, to prevent carry-over of traces of HBr and subsequent premature denitrosation of the N-nitroso compounds. Values reported for the coefficients of variation were 27.9 and 21.1%, respectively, at the levels of spiking of N-nitrosarcosine equivalent to 32.9 ng and 146 ng of nitric oxide.

A method based on the UV decomposition of N-nitroso compounds in the gas phase after their separation by GC, and subsequent determination of the NO· radicals thus produced, by means of a chemiluminescent detector is proposed (Budevska *et al.*, 1986). The photolytic mode has some advantages compared to the pirolytic one, such as, most compounds (non-nitroso, C,O-nitroso) which give false positive reponses with the standard TEA detector, cannot be decomposed by UV light. Therefore, authors concluded that the photolytic chemiluminescence detector can serve as a selective and sensitive technique for the quantification of N-nitroso compounds.

Pinche *et al.* (1989) have applied HPLC with denitrosation and chemiluminescence detection to the separation and individual assay of N-nitrosamines in solution. Nitrite is differentiated from these compounds by carrying out an initial selective reduction. The study is performed on a mixture of four reference N-nitrosamines. Resolution (separation of the four reference N-nitrosamines was achieved in 18 min), linearity and reproducibility of the method are evaluated. Limits of detection in the range 0.6–1.2 nmol (1 nmol injected) were found for the N-nitrosamines studied. Therefore, the authors conclude the method can be applied to the differential assay of N-nitrosamines in pork product and may possibility be of use in samples including liquid and solid foodstuffs without major modifications.

The ability of GC-TEA for the confirmation of the presence of certain N-nitrosamines in foods and food-contact materials have been manifiested (Havery & Fazio, 1985). This system has been employed to study the effect of several factors in the formation of N-nitrosothiazolidine (NTHZ) (Pensabene & Fiddler, 1985), N-nitrosopyrrolidine and N-nitrosodimethylamine (Skrypec *et al.*, 1985) in bacon. In the same way, Hotchkiss & Vecchio (1985) and Hotchkiss *et al.* (1985) studied N-nitrosamine formation in fried-out bacon fat. Further studies were focused on investigation of N-nitrosamines in elastic rubber nettings (Sen *et al.*, 1987a; Sen *et al.*, 1988) and in baby-bottle nipples and baby pacifiers using chromatographic techniques and TEA (Sen *et al.*, 1985*a*; Sen *et al.*, 1987*b*). Sen *et al.* (1987*b*) used propyl gallate as inhibitor for artifactual formation of nitrosamines and extraction with dochlorometane was carried out in an ambient temperature. In a recent paper nitrosodimethylamine has been determined by chemiluminescent detection in a wide variety of Nigerian foods (Atawodi *et al.*, 1993). Levels of between 0·4 and 4·6 ppb were detected in 75% of the samples analyzed.

There is considerably less information about analytical methodology for non-volatile N-nitrosamines.

Some compounds of this type may be associated with the components of the matrix itself and therefore extraction procedures would be inefficient (Walters *et al.*, 1978), although considerable progress has been made in this field. Sen *et al.* (1983) describe a sensitive method for the determination of N-nitrosoproline and N-nitrososarcosine in malt and beer. Previous extraction of the samples with methanol and formation of methyl esther derivatives with diazomethane or BF_3-methanol, the determination is carried out by gas–liquid chromatography using a thermal energy analyzer detector with a highly satisfactory average percentage of recoveries (84–90%). The average levels of N-nitrosoproline detected in 11 malt and 28 beer sample were 24·1 ppb and 1·7 ppb, respectively. The determination of N-nitroso-N-methylurea (NMU), a nitrosamide, in fried bacon (Sen *et al.*, 1985b) was reported by HPLC–chemical denitrosation–TEA system. Extraction with ethyl acetate and later with *n*-hexane is carried out. The minimum detection limit of the method reported was about 10 ppb. The paper also presented developed methodologies for N-nitrosothiazolidine and N-nitrosothiazolidine-4-carboxylic acid in fried bacon and other cured meats.

Assay of nitrite in food samples, in particular pork products, can be carried out by CLD after chemical reduction and recovery of the liberated nitric oxide. Several reduction agents have been used, iodide ion in a weakly acidic medium of aqueous acetic acid (Cernes *et al.*, 1987) or sodium ascorbate in tartaric acid (Fiddler, 1984) were some of them. The effectiveness of the denitrosation method and CLD for determination of nitrite has been compared by Fiddler *et al.* (1984) with the AOAC method of sulfanilamide/N-(1-naphthyl)ethylenediamine reagent. There are no significant differences in the statistical analysis of the results obtained for $NaNO_2$ in pork products (bacon, ham, frankfurter, etc.). Moreover, these authors tried to simplify the sample preparation avoiding the heating step and comparing the results on nitrite with samples heated, concluding that CLD requires this step for maximum nitrite production.

The procedure proposed by Pinche *et al.* (1991) supplies a suitable method for assaying nitrite by direct introduction of the sample (pork products) in the reduction solution (bromide hydrogen in acetic acid medium) at 65°C. Moreover, destruction of nitrite by sulfamic acid prior to the measurements allows the separate determination of nitroso compounds. The authors reported a detection limit for nitrite of 0·1 nmol. Linearity and repeatability (0·87–174 nmol and 1·98–17·3%, respectively) were good and there was no evidence of false positive response. A rapid method for the determination of nitrate and nitrite by chemiluminescence has been proposed (Walters *et al.*, 1987) in order to optimize concentration of reducing agents utilized and its influence on the release of nitric oxide. Sodium iodide in acetic acid and ferrous ammonium sulphate–ammonium molibdate (in proportion 2:1) were used as reducing agents for nitrite and nitrite + nitrate, respectively.

Gas chromatography or high-performance liquid chromatography with modified TEA detection have been employed in the determination of amines in foods with prior derivatization. In this way, a method has been proposed (Pfundstein *et al.*, 1991) for routine determination of primary and secondary amines in foodstuffs. Benzenesulphonyl chloride (BSC) was used in order to obtain the corresponding sulphonamides which were separated in primary and secondary amine derivatives using a modified Hinsberg procedure. The advantages of the TEA detector for the determination of nitrogen-containing compounds were compared for the detection limit with other reported methods.

Fujinari and Courthaudon reported in some papers (Courthaudon & Fujinari, 1991; Fujinari & Courthaudon, 1992) about chemiluminescent nitrogen detector with gas chromatography and its applications to selective detection of nitrogen-containing compounds, e.g. in flavors and essential oils (Benn *et al.*, 1993). Adulteration of a natural peach flavor and of the essential oil of galbanum were investigated. The authors concluded that a linear detector response is obtained and they point out the advantage of the ability to peer through complex sample matrices, thus simplifying the detection, identification and quantification of nitrogen-containing compounds.

Determination of sugars

There is interest in the determination of sugars in food products. The most commonly found sugars are glucose, sucrose, maltose, lactose and fructose. There has been considerable recent interest in combining the advantages of chemiluminescence detection with the specificity of enzyme reactions. Conventional enzymatic determinations involve adding the enzyme to the sample and, after an incubation period, measuring the concentration of a substrate or product.

The glucose reacts with glucose oxidase in the presence of molecular oxygen to produce hydrogen peroxide. The hydrogen peroxide produced is then quantified by reaction with luminol in the presence of a metal ion catalyst to produce light (Petersson *et al.*, 1986). These two reactions occur at very different pHs, which becomes the most serious problem in coupling these two reactions. This fact has been overcome (Koerner & Nieman, 1986) by use of a microporous membrane flow cell in which oxidase enzyme (buffered at its optimum pH) is contained in a reservoir and forced through a microporous membrane into the analyte side where an injected plug containing analyte and chemiluminescence reagents (buffered at the optimum pH for the chemiluminescence reaction) flows past the membrane.

Chemiluminescence provides a sensitive means of detecting the hydrogen peroxide produced by enzyme reactions in a flow system. It offers the advantages of wide linear working ranges, low detection limits, rapid analysis, and instrumentation that is inexpensive and simple to use. Combining chemiluminescence detection

with enzyme reaction provides a method which is both sensitive and selective. With the exception of glucose, the sugars do not react in the presence of an enzyme to produce hydrogen peroxide. However, they can be converted enzymatically to glucose which can then be quantified by the glucose oxidase reaction described before. Koerner & Nieman (1986) determine sucrose in a flow injection system, involving conversion to glucose (via reaction with invertase and mutarotase), generation of H_2O_2 from the glucose (via reaction with glucose oxidase), and the chemiluminescence determination of the H_2O_2 (via reaction with luminol).

The glucose oxidase reaction and chemiluminescence reaction occur in a microporous membrane flow cell. Working range is 5 μM–1 mM with precision of 2–3%. Analysis time is 2 min. To determine sucrose in food products, one must differentiate between glucose originally present and that formed from sucrose. This can be accomplished either by prior determination of the amount of glucose present or by elimination of the glucose prior to the determination of sucrose. Although both approaches give acceptable results, the destruction approach is preferred in terms of both accuracy and convenience. The food products analyzed include soft drinks, breakfast cereal, and cake mix.

Koerner & Nieman (1988) also determine maltose, lactose and fructose by enzymatic conversion to glucose (using amyloglucosidase, lactase, and glucose isomerase, respectively) and subsequent determination of the glucose. All enzymes are immobilized on controlled-pore glass and contained in flow-through reactors. These analytical procedures have certain characteristics which are common to all sugars analyzed. These include minimal sample pretreatment, short analysis times, and good precision. The only sample pretreatment required is dilution of the sample to fall within the linear calibration range. The method described for the determination of glucose, sucrose, maltose, lactose, and fructose thus gives detection limits, working ranges, analysis times, and the precision at least as good as, in fact generally better than, other methods currently in use for the determination of these sugars.

A flow-injection analysis system was developed by Puchades *et al.* (1993) for the determination of lactose in milk. Milk was injected into a carrier stream of phosphate buffer containing EDTA and the stream was passed through a dialysis unit, a β-galactose reactor, and a co-immobilized glucose oxidase–mutarotase reactor to form H_2O_2. A stream of luminol in sodium carbonate converged with a stream of hexacyanoferrate (III) and after mixing, merged with the generated H_2O_2 stream and the resulting chemiluminescence was measured. The calibration graph was rectilinear for 20–200-mM lactose and the RSD ($N = 11$) was 0·49%.

Suleiman *et al.* (1993) present a system consisting of a reagent stream (0·3 ml/min) of 1-mM luminol in 25-mM Na_2CO_3, which was merged with a second stream of 10-mM $K_3Fe(CN)_6$. The sample stream (0·3 ml/min) consisted of 50-mM acetate buffer of pH 5·6 and was passed over a column of immobilized glucose oxidase reactor, which oxidized the glucose present in the sample to gluconic acid and H_2O_2. The latter was transported by the sample stream to a flow cell, where it reacted with the mixed reagent stream. The calibration graph was rectilinear for 0·25–2·5 mM with an RSD of 5%. The cited method was applied to the determination of glucose in baby fruit juices.

Food oxidation

Deterioration of dietary lipids by oxidation reactions is an important topic in food science and nutrition. Lipid peroxidation has received much attention recently in connection with its pathological effects and possible contributions to cancer and aging (Yamamoto *et al.*, 1987). The increasing concern over the possible relationship between lipid peroxidation and certain ailments, and correspondingly with food processing and storage, has prompted the examination of various techniques for the determination of hydroperoxides formed during lipid oxidation. The oxidation of lipids proceeds by a free-radical chain mechanism and gives lipid hydroperoxides as primary products. A sensitive, specific, and accurate method for measuring these hydroperoxides is needed to provide the necessary data to understand the oxidative mechanisms for lipids and to evaluate the efficiency of the many natural and artificial antioxidants present in foods. The luminol/microperoxidase assay has been adapted to organic hydroperoxides and observed a sensitivity similar to that of hydrogen peroxide. Yamamoto *et al.* (1987) assume the following reaction mechanism of lipid hydroperoxides (LOOH) with isoluminol to produce chemiluminescence: LOOH + microperoxidase $\rightarrow$ LO· LO· + isoluminol (QH^-) $\rightarrow$ LOH + semiquinone radical ($QH^{-}\cdot$) $Q^{-}\cdot + O_2 \rightarrow Q + O^{-}_{2}\cdot$ $Q^{-}\cdot + O^{-}_{2}\cdot \rightarrow$ isoluminol endoperoxide $\rightarrow$ light

It has been shown that hydrocarbons and lipids, while being oxidized, emit light. This chemiluminescence could be attributed to different possible steps of the lipid oxidation radical chain. Ultra-weak chemiluminescence has been used by many authors for the investigation of oxidation reactions of foods containing polyunsaturated lipids.

Mazurczak *et al.* (1983) determine the degree of fat rancidity for edible and usable fats by a rapid chemiluminescence method. 1-g feed samples were spread on a 6-cm^2 surface, kept in the dark for 15 min, then placed under a photocathode. The degree of rancidity was determined by measuring the emission. Timms & Roupas (1982) use a scintillation counter to measure chemiluminescence from oxidizing milk fat stored at 20, 50, and 80°C and sunflower oils stored at 50°C. The quimioluminescence for milk fat increases with oxidation after an initial slight decrease, it was higher at higher storage temperatures even though all measurements were made at 20°C. For soy-bean and sunflower oils, chemiluminescence fell with increasing storage time and oxidation. Loeliger & Saucy (1984) have proposed the photon emission from food lipid peroxidation for food

quality determinations. They make a chemiluminescence determination of different foods (potato flakes, wheat cereal, milk powder and lean pork) at 50°C. Higher chemiluminescence counts normally indicate higher degrees of oxidation.

A method for the detection of various hydroperoxides and hydrogen peroxide at the picomole level has been developed by combining an HPLC system with an ultrasensitive analytical system based on the detection of chemiluminescence emitted by luminol in the presence of hydroperoxide and microperoxide. Yamamoto *et al.* (1987) have adapted the luminol/microperoxidase assay to organic hydroperoxides and observed a sensitivity similar to that of hydrogen peroxide. One great advantage of measuring light emission from the oxidation of luminol by lipid hydroperoxides is that it is not necessary to heat the reaction, because the reaction is rapid even at room temperature. They have adapted the assay for post-column HPLC detection, which overcomes the disadvantage of the interference of antioxidants by HPLC, and also makes it easy to identify the nature of the lipid hydroperoxides by the retention time.

Benov & Ribarov (1990) present a chemiluminescence method for the determination of lipid hydroperoxides in cloroform–methanol extracts of food. This method is also based on registration of the luminol-dependent light emission induced by catalyzed decomposition of hydroperoxides. The main advantages of the chemiluminescence method are its specificity and sensitivity. By this method 0·01 nmol of lipid hydroperoxides could be determined. Yang *et al.* (1991) use a reversed-phase liquid chromatography method to separate the hydroperoxides, followed by post-column chemiluminescence detection.

Detection of hormonal anabolics and metabolites

The laws in some countries require the evidence of the presence of residual steroids. Meats that contain residuals of anabolic steroids are considered harmful to the human health. One of the most commonly employed is the 19-nortestosterone (nandrolone). Jansen *et al.* (1984), Jansen *et al.* (1985), and Jansen *et al.* (1989) developed inmunologic methods employing chemiluminescent labels. The described procedures are highly selective and powerful in investigations for the presence of anabolic steroids in urine, and in parts of animals for human consumption. The chemiluminescent detection is carried out by its high sensitivity and rapid measurement. The combination of the chemiluminescence immunoassay (CLIA) with HPLC provides a highly selective method.

A specific identification method for a number of hormonal anabolics, especially 19-nortestosterone, methyltestosterone and zeranol and their metabolites, is based on a combination of selective fractions by HPLC and immunochemical detection with chemiluminogenic steroid/isoluminol conjugates (Jansen *et al.*, 1985). N-(4-Aminobutyl)-N-ethylisoluminol (ABEI) was prepared. 17α-Methyltestosterone (MT) and 19-nortestosterone (NT) 3-carboxymethyloxime derivatives and zeranol (Z) 7-carboxymethyl derivative (zearalanon) were coupled to ABEI via the N-hydroxysuccinimide esther to produce the chemiluminescent labels. Samples of bovine urine were purified after enzymatic hydrolysis by ether extraction. An aliquot of urine was applied to the HPLC column. The dry HPLC fraction of a urine extract was dissolved and incubated with a solution of the corresponding ABEI label and the corresponding antiserum. After addition of microperoxidase solution the chemiluminescence was initiated by addition of hydrogen peroxide.

Van den Berg *et al.* (1988) present a solid-phase chemiluminescence immunoassay for 19-nortestosterone (NT). NT-3-carboxymethyloxime/N-(4-aminobutyl)-N-ethylisoluminol serves as the label with an antiserum raised against NT-3-carboxymethyloxime/bovine serum albumin. This assay can be used generally for the detection of anabolic agents in application sites. Because of the high sensitivity (0·1 pg NT/tube at 90% relative binding), only 250 μg of muscle tissue is necessary for the assay. Van Peteghem (1986) describes the isolation and clean-up of 19-NT from tissue samples, previously the chemiluminescent immunoassay for 19-NT described by Jansen *et al.* (1984). A sample of minced meat was deproteinated enzymatically. The liquid suspension was chilled to room temperature and extracted with diethylether. The combined layers were evaporated to dryness. The crude extract was taken up in hexane–dichloromethane and applied on top of a small glass column plugged at the bottom with glass wool and filled with Lipidex-5000. The residue obtained after evaporation of Lipidex-5000 fraction was taken up in methanol and transferred to a vial suited for HPLC. The 19-NT-containing fraction was evaporated to dryness. Aliquots of the final extract were subjected to CLIA as described by Jansen *et al.* (1984).

Van Peteghem & Van Look (1988) present a comparison of chemiluminescence immunoassay (Van Peteghem, 1986) to a radioimmunoassay, which involves the same clean-up procedure but makes use of ^{3}H-labeled nortestosterone as the tracer and an antiserum raised against a different immunogenic form of the analyte. Both methods have a similar sensitivity but the limit of quantification is much lower for a radioimmunoassay (0·08 μg kg^{-1}) than for chemiluminescence immunoassay (0·6 μg kg^{-1}). Jansen *et al.* (1989) examined a steroid solid-phase immunoassay with peroxidase labels using two detection methods. Immunoassays were applied for 19-nortestosterone (NT) by using a solid phase coated with a second antibody and a solid phase coated directly with a NT-antibody. For end-point detection, either an enzyme-enhanced chemiluminescence procedure or spectrophotometry based on tetramethylbenzidine was used. Both methods gave similar calibration graphs. The chemiluminescence procedure was more sensitive and the second-antibody immunoassays provided lower limits of detection.

Determination of some metals

Recently, some authors have investigated the determination of metals in several samples. The determination of nickel in water was investigated by Lu *et al.* (1991). The nickel can act as a catalyst in the chemiluminescence system of acetone–H_2O_2–ClO^-, and the catalyzed chemiluminescence intensity is proportional to the concentration of Ni^{2+}. The limit of detection for Ni^{2+} by this method is 2·5 ng ml^{-1}, and the linear dynamic range is from 10 ng ml^{-1}. Lu *et al.* (1993) propose a method for monitoring chromium in environmental water, based on the reaction of H_2O_2/luminol in an alkaline medium. The water was passed through an anion exchange column to determine Cr(III) and the sample was passed throught a cation exchange to determine Cr(VI). The calibration graphs were rectilinear for 10–10000 ng of Cr with a detection limit of 40 pg/ml and RSD < 2%.

An indirect chemiluminescence method for determination of trace of Mo (VI) is described for Zhang *et al.* (1988). This method utilizes the reaction of I^- with H_2O_2, accelerated by Mo(VI) to produce I_2 and I_2 oxidize the luminol to produce chemiluminescence. The detection limit is 6×10^{-10} g/ml Mo. The linear range of the calibration curves is 1×10^{-9} to 1×10^{-7} g/ml Mo. The relative std. deviation is < 6%. This method has been used for the determination of trace Mo in the cereal grains.

Flow injection (FI) has been applied to the determination of Cr(III) in water and food samples (Escobar *et al.*, 1993). The method is based on the measurement of light emitted from the Cr(III)-catalyzed oxidation of luminol by H_2O_2. The specificity of this reaction for Cr(III) can be achieved in the presence of ethylenediamine tetraacetic acid (EDTA). The luminol–H_2O_2 chemiluminescence reaction is catalyzed by Cr(III) and other metals, and the addition of EDTA greatly reduces the luminescence of the reaction because of the formation of metal–EDTA complexes. The Cr(III)–EDTA complex is thermodynamically stable but kinetically slow to form at room temperature, whereas all the other metal ions that catalyse the luminol–H_2O_2 reaction rapidly form complexes with EDTA. The apparatus consists of an FI system with a flow cell suitable for chemiluminescence detection. The flow cell, situated near the photodetector, is a coiled tube made from transparent poly(tetrafluoroethylene). The detection limit is 0·01 ppb and the linear range extends up tp 6 ppb. The system was shown to possess a high selectivity. The proposed method was applied to the analysis of several samples of water (distilled water, mineral water, drinking water and polluted water) and food (brown bread, shrimp, bovine muscle and brewer's yeast). The data obtained for the analysis of different types of food samples coincide with the values obtained for Cr(III) in food reference materials of this type.

Detection of gamma-irradiated foodstuffs

Chemiluminescence seems to be a useful technique for the identification of irradiation in foodstuffs (Sjoeberg *et al.*, 1990). Luminol, mainly and lucigenin solutions can be used as chemiluminescent reactions. Boegl & Heide (1985) reported that chemiluminescent measurements were succesfully used as an indicator for exposure gamma radiation doses up to 10^4 Gy of several foods (milk powder, whole onions, differents spices, frozen chicken) using a luminol solution to form the chemiluminescence. Possibilities in distinguishing between chemiluminescence caused by UV irradiation and that caused by gamma radiation are dicussed.

Most papers focus on studying the effects of different parameters on chemiluminescent response, e.g. irradiation doses and particle size on chemiluminescence of pepper and of irradiation on chemiluminescence of papain have been studied (Sattar *et al.*, 1987). A comparative study (Chang Ma *et al.*, 1992) between unirradiated and irradiated samples of 10 agricultural products including wheat flour, rice, ginger, potatoes, garlic, onions, red beans, mung-beans, soy-beans, xantoxylon seed and japanese star anises reported no significant differences between treated and non-treated samples, except onion bulb and wheat flour. Effects of protein content (high, medium, low), particle size, the reproducibility and the storage at different irradiation doses (0–10 hGy) were reported. Authors concluded that chemiluminescent measurements could be used as good indicators for the identification of irradiated wheat flour within a post-irradiation storage time of 7 days. There are wide fluctuations in the data reported regarding the utility of chemiluminescent measurements in this area, even among samples studied.

Quantification of other compounds

Determination of alcohols in beverages and food.
The method involves treating test alcohol in a sample with alcohol oxidase and measuring H_2O_2 formed with chemiluminescence assay. This method was proposed for Fukuoka *et al.* (1991), determining the H_2O_2 treating the sample with luminol and K ferricyanate in a vial and counting the chemiluminescence. The detection range was $1–10^4$ ppb.

Determination of enzymes.
Puchades *et al.* (1994) have determined peroxidases and lactoperoxidase in vegetables and dairy products. The chemiluminescence method involves the peroxidase-catalyzed reaction of H_2O_2 with luminol.

CONCLUSIONS

CL reactions have considerable analytical potential because they have numerous advantages: high sensitivity (for many compounds detection limits of 1 μg/kg or lower have been reported), wide linear range and the use of simple and inexpensive instrumentation for monitoring emission, where the absence of a light source reduces the background noise. All these advantages have allowed the method that has been conveniently used in

the determination of many inorganic and organic compounds in food samples. There is ample documentation that CL detection in combination with chromatographic techniques becomes highly selective, i.e. determination and identification of volatile nitrosamines or lipid hydroperoxides. In this way, CLIA in combination with HPLC has demonstrated to be an excellent and fast screening method for detection of hormonal anabolics. The combination of sensitivity of CL with rapidity of FI, together with low cost and simplicity, make the FI-chemiluminescence system extremely attractive, especially to determine sugars in food. Although it may be assumed that CL measurements could be used as a useful indicator for the identification of irradiated foodstuffs, several disadvantages, particularly in the extent of uncertainties for some foodstuffs, suggest that further research is needed to establish the reliability of this method for this purpose. The above discussion of CL in food analysis shows the reader the variety of excellent methods in this area, providing important and fascinating fields of investigation. However, a more systematic optimization of several systems is still necessary. In spite of that, we conclude that the CL techniques can be used for the routine analysis of complex food samples in many laboratories.

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