Recent analytical applications of nanoparticle sensitized lucigenin and luminol chemiluminescent reactions

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Abstract. There is an ever-increasing demand for rapid, sensitive, cost effective and selective detection methods for the analysis of many essential compounds. When chemiluminescence has been introduced to analytical chemistry as a detection technique, it has been shown to meet many of these requirements. This method has become a powerful tool for the determination of many compounds. Using this method, low detection limits can be obtained with simple and inexpensive instrumentation. Coupled with flow injection technique the method has become more popular for wider applications. Since many excellent reviews on the chemiluminogenic techniques have appeared in the literature in recent years, the present paper does not intend to cover the exhaustive studies in this area, but will selectively describe the analytical applications of nanoparticle sensitized lucigenin and luminol chemiluminescent reactions and evaluate their recent progress together with our present work.

Keywords. Chemiluminescence; flow injection analysis; lucigenin; detection methods.

1. Introduction

An emission of electromagnetic radiation (ultraviolet, visible, or infrared) produced by molecules that are electronically excited during the chemical reaction is known as chemiluminescence (CL). The decay of the excited state to a lower energy level is responsible for the emission of light. As the emission of light is a function of the concentration of the chemical species involved in the chemiluminescence reaction, measurement of emission intensities can be used for analytical purposes. Chemiluminescence differs from fluorescence in that the electronic excited state is derived from the product of a chemical reaction rather than the more typical way of creating electronic excited states, viz. absorption. It is the antithesis of a photochemical reaction, in which light is used to drive an endothermic chemical reaction. Here, light is generated from a chemically exothermic reaction. A standard example of chemiluminescence in the laboratory setting is the luminol test. Here, blood is indicated by luminescence upon contact with iron in hemoglobin. When chemiluminescence takes place in living organisms, the phenomenon is called bioluminescence. A light stick emits light by chemiluminescence. Chemiluminescent techniques can be applied in two types of analytical methods: direct method and indirect method. In direct method, a substrate and an oxidant in the presence of some cofactors and catalyst react to yield a product or intermediate. A fraction of the product or intermediate is formed in an electronically excited state and

subsequently goes to the ground state with emission of light and in indirect method, this process involves transfer of energy of the excited species to a fluorophore like terbium sensitized chemiluminescence and post chemiluminescence.

The most popular chemiluminescent systems include luminol and lucigenin. The important features of chemiluminescent systems are good sensitivity, simple instrumentation, low detection limit, wide range of applications and large linear range of analytes. Because of these advantages, chemiluminescence occupies a position as one of the more sensitive techniques of trace analysis of essential compounds. Alves *et al* (2003) established a luminal and a lucigenin dependent chemiluminescence methods to investigate the role of receptors for Fc portion of IgG (FcγR) and/or complement receptors (CR) in mediating the oxidative burst in neutrophils from systemic lupus erythematosus (SLE) patients compared with healthy controls. In the luminal–CL system, all the reactive oxygen species (ROS) are responsible for light production, whereas in the lucigenin–CL system, only the first ROS generated, converts the lucigenin into an unstable intermediate molecule, which also emits light. First, neutrophils from healthy controls and SLE patients were stimulated with different IC opsonized or not with complement from normal human serum (NHS) or SLE serum, in presence of 10^{-4} M luminol. To improve the sensitivity of chemiluminescence detection, metal catalyzed chemiluminescence has been extensively applied to bioanalysis and immunoassays. Most recently nanoparticles are frequently used as they display unusual catalytic properties for a number of chemical reactions, depending upon their size, shape and stabilizing agents. However, the applicability of nanoparticles as catalysts in chemiluminescence reaction is

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still unknown. It was found that lucigenin alkaline solution could react with hydrazine in the presence of platinum nanoparticles to generate strong CL centred at 480 nm. It is suggested that the CL enhancement might be due to the fact that thiol-containing compounds could facilitate the electron transfer process under the catalysis of Pt nanoparticles and accelerate the generation of OH $\cdot$ and O $_2^{\cdot-}$ radicals, leading to the strong CL (Liu *et al* 2011). A CL system was developed for the detection of fluorescent organic compounds (FOCs). The CL system is composed of an oxidizing agent, peroxomonosulfate (KHSO $_5$) and a metal ion catalyst, cobalt (as sulfate). In unbuffered conditions, HSO $_5^-$ is decomposed by Co $^{2+}$ to yield reactive species (Co $^{3+}$, SO $_4^{\cdot-}$, etc), which are strong oxidizing agents leading to CL from FOCs (Tsukada *et al* 1998). Chen *et al* (2007) found that silver nanoparticles (AgNPs) could enhance the CL intensity of luminol–H $_2$ O $_2$ system. In this reaction, luminol intermediate is generated under alkaline condition on the surface of AgNPs in luminol–H $_2$ O $_2$ system and enhances CL intensity.

Since a number of reviews on the chemiluminescence techniques in analytical chemistry have appeared in recent years, the present paper does not intend to cover the exhaustive research on this area, but selectively describe key references and recent advances together with our current work. Recent trends will be focused in the last section on the use of nanoparticles for significant improvements in CL detection systems.

2. Analytical applications

Chemiluminescence (CL) analysis has been frequently used for the analysis of organic and inorganic species because of its low detection limit, wide dynamic range and simple instrumentation. Recently, Du *et al* (2007) used lucigenin CL reaction with flow injection technique for the determination of Cr(III) and total Cr. Strong chemiluminescence signal was produced from the reaction of Cr(III), lucigenin and KIO $_4$ in alkaline media. The determination of total Cr was carried out by prereduction of Cr(VI) to Cr(III) by using H $_2$ SO $_3$. The detection was reported here (1×10^{-10} g/ml). To test the applicability of the method water sample was analysed for Cr(III) and total Cr.

Determination of epinephrine using electrochemiluminescence (ECL) of lucigenin system by the anodic potential sweep had been performed (Su *et al* 2005). ECL response of lucigenin was found at anodic potential above 1.1 V (vs Ag/AgCl) in non-ionic surfactant Triton X-100 and the ECL signal was significantly enhanced by epinephrine. Under optimum condition, enhanced ECL intensity was linear with the epinephrine concentration in the range of 4.0×10^{-8} to 2.0×10^{-7} M and the detection limit (defined as $S/N = 3$) was found to be 2.4×10^{-8} M. The method was successfully applied to the determination of epinephrine in pharmaceutical samples with satisfactory results. The reaction mechanism for the lucigenin ECL system in the presence of epinephrine was also discussed.

Li and Lee (2005) developed a new chemiluminescence (CL) method for the selective determination of As(III) and As(V) ions in aqueous solution using a FIA system. The method was based on the increased CL intensity with the addition of As(V) ion into a solution of lucigenin and hydrogen peroxide. The addition of As(III) ion into the solution did not change CL intensity. Total concentration of As ions was determined after pre-oxidation of As(III) to As(V) with hydrogen peroxide in basic solution. The As(III) content was estimated by subtracting the content of As(V) ion from total As concentration. The effects of concentrations of KOH and H $_2$ O $_2$, and flow rates of reagents on CL intensity had been investigated. The calibration curve for As(V) ion was linear over a range from 1.0×10^{-2} to 10 μ g/g, the coefficient of correlation was 0.997 and the detection limit was 5.0×10^{-3} μ g/g under the optimal experimental conditions. Lucigenin has been used to determine catechin in aqueous solution by measuring chemiluminescence intensities using a stopped flow system (Lee *et al* 2005). The lucigenin–hydrogen peroxide chemiluminescence reaction was chosen for the determination of catechin. Fe(II) ion was added to the chemiluminescence system to enhance the CL sensitivity. The chemiluminescence intensity from the lucigenin system was increased by the addition of catechin. Effects of flow rates of reagent and sample and concentrations of lucigenin, hydrogen peroxide, Fe(II) ion and KOH were studied. The calibration curve for catechin was linear over a range from 1.0×10^{-6} to 1.0×10^{-3} M and the detection limit was 3.0×10^{-7} M under optimal experimental conditions.

Our group developed CL method combined with the flow injection analysis (FIA) technique (Lee *et al* 2007) for the quantification of bilirubin based on the quenching effect of bilirubin on CL from the lucigenin–hydrogen pe-

Figure 1. CL kinetic curves of system (I), lucigenin–H $_2$ O $_2$ in absence of bilirubin, and system (II), lucigenin–H $_2$ O $_2$ in presence of bilirubin (Conditions: lucigenin, 5×10^{-4} M; bilirubin, 58.47 μ g/ml; H $_2$ O $_2$, 2.5 M; NaOH, 5 M; flow rate, 3.0 ml/min; sample volume, 200 μ l (adapted from Chen *et al* (2011)).

Figure 2. Schematic diagram of FIA-CL manifold employed for quantitative determination of bilirubin. P_1 , P_2 , peristaltic pumps; T_1 , T_2 , Y-pieces (adapted from Chen *et al* (2011)).

roxide system in an alkaline medium (figure 1). The flow-injection analysis (FIA) set up used in this study is shown in figure 2. Under the optimum conditions, decreased CL intensity was proportional to the concentration of bilirubin, in the range 0.0585–58.47 $\mu\text{g/ml}$. The detection limit estimated from the calibration graph was about 7.8826 $\mu\text{g/ml}$. The relative standard deviation (RSD) of 10 parallel measurements (1×10^{-4} M bilirubin) was 2.5%. Recoveries of bilirubin were found to fall in the range 94–97.5% using control sera. The method is interference-free, fast and easy to carry out.

Concerning the reaction mechanism, the CL inhibition by bilirubin of the lucigenin– H_2O_2 reaction was proposed to be due to the competition with lucigenin for hydrogen peroxide. Bilirubin reacted with oxidant H_2O_2 to partly consume H_2O_2 , forming biliverdin. Thus, bilirubin inhibited the CL intensity of the lucigenin– H_2O_2 –NaOH system. This phenomenon was confirmed by a preliminary UV-vis spectroscopic study. From the study it was observed that bilirubin had a maximum absorption peak at 434 nm. Addition of H_2O_2 to bilirubin resulted in a gradual change of the bilirubin yellow colour to the green biliverdin, and the biliverdin peak appeared at 291 nm simultaneously with the diminution of the bilirubin peak at 434 nm.

A simple continuous-flow CL system was reported by Li *et al* (2007), for simultaneous determination of glucose, fructose and lactose in ternary mixtures of reducing sugars without previous separation. The method was based on the different kinetics of the individual sugars in the oxidation reaction with potassium ferricyanide. The known luminol– $K_3Fe(CN)_6$ CL system was used to measure the kinetic data of the system. The CL intensity was measured and recorded every second from 1 to 300 s. The data obtained

were processed chemometrically using an artificial neural network. The relative standard errors of prediction for three analytes were <5%. The proposed method was successfully applied to the simultaneous determination of three sugars in some food samples.

A flow-injection procedure was reported for the determination of carbaryl based on its inhibition effect on the chemiluminescence from luminol–cobalt(II) system in alkaline medium in the presence of hydrogen peroxide (Waseem 2007). The calibration curve was found over a range 5.0×10^{-7} to 20×10^{-6} M and the detection was 2.37×10^{-7} M for carbaryl. The effects of some carbamates, anions, and cations were investigated on luminol CL system for the determination of carbaryl. The proposed method was applied for the assay of carbaryl in natural waters.

Li *et al* (2007) reported a flow injection chemiluminescence method for the determination of four phenothiazine drugs, such as chlorpromazine hydrochloride, perphenazine hydrochloride, fluphenazine hydrochloride and thioridazine hydrochloride. Intense CL signal was produced when the drug was injected into the mixed stream of luminol with $KMnO_4$. The dynamic ranges of the method were 0.0020–1.0 $\mu\text{g/ml}$ chlorpromazine hydrochloride, 0.0040–3.0 $\mu\text{g/ml}$ perphenazine hydrochloride, 0.0020–5.0 $\mu\text{g/ml}$ fluphenazine hydrochloride and 0.0050–1.0 $\mu\text{g/ml}$ thioridazine hydrochloride. The detection limits were found to be 0.4 ng/ml chlorpromazine hydrochloride, 0.7 $\mu\text{g/ml}$ perphenazine hydrochloride, 2 $\mu\text{g/ml}$ fluphenazine hydrochloride and 0.7 $\mu\text{g/ml}$ thioridazine hydrochloride.

Picogram-level gatifloxacin was determined by Xie *et al* (2007) which was based on its significantly catalytic effect on myoglobin–luminol CL reaction in FIA system. The

enhanced chemiluminescence intensity was proportional to the concentration of gatifloxacin in the range from 50 $\mu\text{g/L}$ –10 $\mu\text{g/L}$, and the detection limit was 20 mg/L . The method was applied to the determination of gatifloxacin in tablets, human serum and urine samples with the recovery from 97.4–104.5%. DNAzyme-functionalized Au–NPs act as catalytic labels for the amplified detection of DNA and telomerase activity on nucleic acid-functionalized gold surface. The DNAzyme stimulates, in the presence of hemin, H_2O_2 , and luminol, the generation of chemiluminescence (Niazov *et al* 2004). It was found that Au–Ag alloy nanoparticles could enhance the chemiluminescence of rhodamine 6G–cerium(IV) system, and the most intensive CL signals were obtained by Au–Ag alloy nanoparticles with a molar ratio of 3:4 (Li *et al* 2009a, b, c). Sau *et al* (2001) addressed the gold particle catalyzed eosin reduction in the presence of NaBH_4 . The catalytic activity of the gold nanoparticles is different for different size regimes (size domain dependent) of the particles. It has been shown that the kinetics of the catalytic rate has a bearing with the particle size (*viz.* surface area, mass, etc). The kinetics of catalytic reduction revealed that for the same surface area of different particles, the catalytic rate does not increase proportionately with the decrease in size over the whole range of average diameter from 10 to 46 nm. The effect of ZnO nanoparticles on the luminol– H_2O_2 CL system, a popular model CL system, was investigated. It was found that ZnO nanoparticles with a size regime from 6 to 21 nm could enhance CL of the luminol– H_2O_2 system, and the strongest CL signal was obtained when the mean diameter of ZnO nanoparticles was 6 nm (Li *et al* 2009a–c). It was found that gold nanoparticles with different sizes could enhance the CL of the luminol– NaIO_4 system in alkaline solution. The most intensive CL signals were obtained with gold nanoparticles having a diameter of 4 nm and CL intensity increased linearly with the concentration of gold nanoparticles (Li *et al* 2008). It was found that gold nanoparticles of small size (<5 nm) could inhibit the CL of the luminol–ferricyanide system, whereas gold nanoparticles of large size (>10 nm) could enhance this CL, and the most intensive CL signals were obtained with 25 nm diameter gold nanoparticles (Guo and Cui 2007). The CL intensity of the luminol– KIO_4 system is strongly enhanced by addition of zinc sulfide nanoparticles (ZnS–NPs) capped with 3-mercaptopropionic acid. On injection of fluoroquinolones into the luminol– KIO_4 –ZnS–NP system, CL intensity is substantially increased. The CL emission peaks at 430 nm, thus indicating that the luminophore is 3-aminophthalate (Li *et al* 2009a–c). It was found that noble metal nanoparticles including Ag, Au and Pt nanoparticles in the presence of adsorbates such as iodide ion, cysteine, mercaptoacetic acid, mercaptopropionic acid, and thiourea could reduce lucigenin (*bis-N*-methylacridinium) to produce chemiluminescence (Xu and Cui 2007). Platinum colloids were found to enhance the CL of the luminol– H_2O_2 system, and the most intensive CL signals were obtained with citrate-protected Pt colloids synthesized with citrate as both a reductant and a stabilizer (Cui *et al* 2005). Zhang

et al (2006) found that potassium permanganate (KMnO_4) could react with gold nanoparticles in a strong acid medium to generate particle size-dependent CL. For gold nanoparticles with a size of 2.6 or 6.0 nm, the reaction was fast and could produce the excited state, Mn(II), with light emission around 640 nm. For gold nanoparticles larger than 6 nm, no light emission was observed due to a much slower reaction rate. The CL intensity was found to increase linearly with the concentration of 2.6 nm gold nanoparticles. The reaction of gold nanoparticles with a potassium periodate–sodium hydroxide–carbonate system undergoes chemiluminescence with three emission bands at 380–390, 430–450, and 490–500 nm, respectively. It was found that the light intensity increased linearly with the concentration of gold nanoparticles, and CL intensity increased dramatically when the citrate ions on the nanoparticle surface were replaced by SCN^- (Cui *et al* 2005). The mixtures of silver(I) and citrate that are used to produce silver nanoparticles evoke intense chemiluminescence with *tris*(2,2'-bipyridyl)ruthenium(II) and cerium(IV), which can be exploited for the determination of citrate ions and other analytes over a wide concentration range (Gorman *et al* 2007). Zhang *et al* (2002) observed chemiluminescence on introducing ethanol vapour to the surface of nanosized ZrO_2 and this effect could be utilized to design a sensor for trace ethanol detection. Cui and Dong (2006) indicated that the strong ECL signals of lucigenin system could be obtained on a gold nanoparticle self-assembled gold electrode both in alkaline and neutral solutions, which is even stronger than that of luminol system on a gold nanoparticle self-assembled gold electrode under optimization conditions. Moreover, the gold nanoparticle self-assembled gold electrode also exhibits good stability for the lucigenin ECL system, overcoming the problem of electrode fouling on a traditional electrode. It was found that lucigenin alkaline solution could react with hydrazine in the presence of Pt nanoparticles to generate strong CL centred at 480 nm. The *bis*-acridinium dication lucigenine was successfully used as a molecular assembler in the functionalization of metal nanoparticles to facilitate approach of the pesticide to the metal surface. From the surface-enhanced Raman scattering (SERS) spectra valuable information about the interaction mechanism between the pesticide and lucigenin can be deduced. In fact, endosulfan undergoes an isomerization upon adsorption onto the metal, while the viologen undergoes a rotation of the acridinium planes to better accommodate the pesticide molecule. An interaction between the N atom of the central acridinium ring and the pesticide $\text{Cl}-\text{C}=\text{C}-\text{Cl}$ fragment is verified through a charge-transfer complex. Sensitive detection of trace bisphenol A (BPA) in water samples has been accomplished via inhibition of luminol CL by BPA on the silver nanoparticles (AgNPs)-enhanced luminol– KMnO_4 CL system for the first time. Under the optimized experimental conditions, CL intensity was found to be proportional to the concentration of BPA ranging from 1.0×10^{-8} to $5.0 \times 10^{-5} \text{ g/L}$ (Chen *et al* 2011). The chemiluminescence immunoassay can be used for determining various analytes in samples. The probe and the assay can be applied

to clinical diagnosis and treatment, pharmaceutical analysis, food safety inspection and environmental monitoring.

3. Recent progress and conclusions

A promising trend is to apply this sensitive chemistry in other techniques like liquid chromatography and capillary electrophoresis. One of the most sensitive and selective detection techniques for chromatography is chemiluminescence (CL), in which the emission of electromagnetic radiation (ultraviolet, visible or infrared) produced by a chemical reaction based on the decay of an excited species to the electronic ground-state is measured. A luminol chemiluminescence (CL) reaction was applied for the simultaneous determination of nine corticosteroids (triamcinolone, prednisolone, prednisone, cortisone, betamethasone, dexamethasone, corticosterone, flumethasone and triamcinolone acetonide) using flow injection. The luminol solution was made in NaOH with hexacyanoferrate(II). Hexacyanoferrate(III) was used as an oxidant. Adequate isocratic chromatographic separation was obtained using a simple mobile phase (acetonitrile–water). The kinetic aspects of chemiluminescence reactions are very important in the sense that maximum intensity occurs simultaneous to the arrival of the substance in the detection cell. Under the optimal experimental conditions, the lowest range injected was 15 mg/l for prednisolone, prednisone and cortisone, and the highest was 2 mg/l for betamethasone. Reproducibility and repeatability ranged from 2.59 to 7.59 and 1.50 to 3.61 for different corticosteroids, respectively.

Capillary electrophoresis (CE) is an advanced analytical technique. It has advantages of simplicity, high efficiency, low cost and short analysis time. CE with electrochemical detection or UV detection has already been applied for determination of a number of compounds but the major drawback of CE with UV detection is its sensitivity. As chemiluminescence (CL) detection is one of the most sensitive detection technologies and the costs of the instrumental set up are relatively low, CL detection has become an attractive detection tool for sensitive detection in capillary electrophoresis. Zhao *et al* (2007) reported a rapid and simple method using capillary electrophoresis (CE) with CL detection for the determination of levodopa. This method was based on enhancement effect of levodopa on the CL reaction from luminol and potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$) in alkaline aqueous media. The optimized conditions for the CL detection were 1.0×10^{-5} M luminol added to the CE running buffer and 5.0×10^{-5} M $K_3[Fe(CN)_6]$ in 0.6 M NaOH solution introduced post column. Under optimized conditions, the linear range from 5.0×10^{-8} to 2.5×10^{-6} M, and the detection limit of 2.0×10^{-8} M for levodopa were obtained.

Recently, metal nanoparticles have been investigated as effective catalysts for the chemiluminescence reaction. Zhang *et al* (2005) observed that gold nanoparticles with a size regime from 6 to 99 nm could enhance CL from the

luminol– H_2O_2 system and the chemiluminescence enhancement by gold nanoparticles of the system was supposed to produce from the catalysis of gold nanoparticles, which facilitated the radical generation and electron-transfer processes taking place on the surface of the gold nanoparticles.

It was reported that gold nanoparticles with different sizes enhance the CL of the luminol– $NaIO_4$ system in alkaline solution. Gold nanoparticles having a diameter of 4 nm give most intensive CL signals (Li *et al* 2008) and CL intensity increased linearly with the concentration of gold nanoparticles. Catechol, hydroquinone and resorcinol were found to quench the chemiluminescence intensity from the luminol– $NaIO_4$ reaction catalyzed by gold nanoparticles, which made it applicable for the determination of these polyphenols. Under the optimum experimental conditions, the detection limits were in the range of 2.1×10^{-9} g/ml to 1.0×10^{-10} g/ml. The method was successfully applied to the determination of catechol in tap water and synthetic samples.

In conclusion, nanoparticle sensitized chemiluminescence has a promising future in analytical chemistry. Versatility of CL–nanoparticle will continue to be exploited for an increasingly diverse range of analytical determinations, especially in biomedical applications and immunoassay.

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