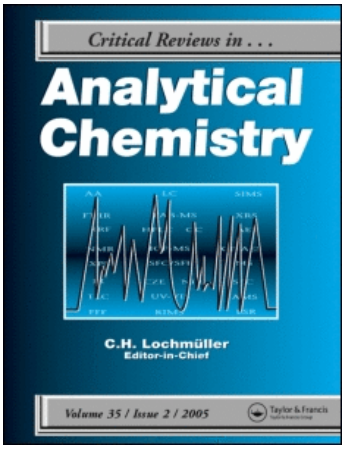

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Recent Developments and Applications of Chemiluminescence Sensors

Hassan Y. Aboul-Enein[a]; Raluca-Ioana Stefan[b]; Jacobus F. van Staden[b]; Xinrong R. Zhang[c]; Ana M. Garcia-Campana[d]; Willy G. Baeyens[e]
[a] Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia [b] Department of Chemistry, University of Pretoria, Pretoria, South Africa [c] Department of Chemistry, Tsinghua University, Tsinghua Yuan, Beijing, P.R. China [d] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Granada, Spain [e] Department of Pharmaceutical Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium

To cite this Article Aboul-Enein, Hassan Y. , Stefan, Raluca-Ioana , van Staden, Jacobus F. , Zhang, Xinrong R. , Garcia-Campana, Ana M. and Baeyens, Willy G.(2000) 'Recent Developments and Applications of Chemiluminescence Sensors', Critical Reviews in Analytical Chemistry, 30: 4, 271 — 289
To link to this Article: DOI: 10.1080/10408340008984161
URL: http://dx.doi.org/10.1080/10408340008984161

Critical Reviews in Analytical Chemistry, 30(4):271–289 (2000)

Recent Developments and Applications of Chemiluminescence Sensors

Hassan Y. Aboul-Enein,[1] Raluca-Ioana Stefan,[2] Jacobus F. van Staden,[2] Xinrong R. Zhang,[3] Ana M. Garcia-Campana,[4] and Willy R. G. Baeyens[5]*

[1] *Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia;* [2] *Department of Chemistry, University of Pretoria, 0002. Pretoria, South Africa;* [3] *Department of Chemistry, Tsinghua University, Tsinghua Yuan, 100084, Beijing, P.R. China;* [4] *Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Campus Fuentenueva, E-18071 Granada, Spain;* [5] *Department of Pharmaceutical Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium;*

ABSTRACT: Chemiluminescence sensors are important tools in analytical chemistry due to their high sensitivity selectivity. This review presents the instrumentation involved in their design, including light detection and flow injection analysis system used. Various applications for the analysis of inorganic and organic compounds from gaseous samples and solutions indicate that these sensors are used with good reproducibility and selectivity of the analytes at low concentration level.

KEY WORDS: chemiluminescence sensors, immunosensors, microbial sensors, flow injection analysis applications.

TABLE OF CONTENTS

* Author to whom correspondence should be addressed. E-mail: enein@kfshrc.edu.sa

1040-8347/00/$.50

I. INTRODUCTION

Chemiluminescence (CL) is well known for its high sensitivity (10^{-6} to 10^{-15} g) and for the low detection limit (10^{-18} g) that can be obtained. A lack in its selectivity is recorded, especially when it is used directly, unlike the spectrometric technique. To increase its selectivity, CL sensors are proposed. Furthermore, the utilization of CL biosensors and CL immunosensors increased the selectivity of CL-based sensors considerably.[1,2] If one compares the selectivity of CL sensors with that achieved by potentiometric or amperometric sensors, it can be concluded that the CL sensors are the most selective ones.[3]

For complex matrices, a separation step cannot be avoided, even if a biochemical reaction is involved and the CL sensor is the most selective one.[4] Moreover, CL emission intensities are sensitive to a variety of environmental factors, such as temperature, solvent, ionic strength, pH, and other species present in the matrices.

Significant advances in design and applications of CL sensors and biosensors were recorded in the last few years.[5] The most utilized type sensor is the flow-through one.[6] The reliability of this type of sensors made them suitable for utilization as detectors in FIA systems.[7]

Due to their electronic structure, some metals (e.g., Ru, Ce) can be used easily for

the construction of sensors.[8-10] However, the CL of luminol is favored by an alkaline medium in the presence of certain ions or molecules (e.g., MnO_4^{2-}, I_2, $[Fe(CN)_6]^{3-}$, Cu^{2+}, OCl^-). It was found that the unsaturated complex of Cu(II) with proteins had a much stronger catalytic effect on the luminol–H_2O_2 CL reaction than Cu(II) alone; this principle was used for the assay of proteins.[11] For the measurement of light, it was proven that the optic system based on fiber optic is the best: multifunctional optical fiber spectrometer, due to its high reproducibility and precision.[5, 12]

II. INSTRUMENTATION

The evolution of instrumentation used for CL sensors is interconnected with the developments achieved for CL instruments (luminometers) and for sensor construction. Taking into account that most of the sensors are based on flow-through sensor follows that the evolution of instrumentation involves also flow systems (cells, pump, valves, tubing, its design, etc.). Therefore, the evolution of CL sensors is related to light detection and to the flow injection analysis system used.

A. Light Detection

CL reactions are very sensitive because the detection of light is of main importance because its sensitivity will directly influence the sensitivity of the CL sensor. Recently, more attention was paid to the evolution of luminometers.[13-15] The photomultiplier tube is still the most used instrument for light detection.[16-18] The utilization of a photodiode proved to give a better sensitivity for light detection.[18]

The sensitivity can be increased by the utilization of a videocamera-based luminograph.[19] It consists of a luminograph (LB 980, EG & G. Berthold, Bad Wildbad, Germany), which is a high-performance low-light imaging system able to detect any type of luminescent emission (400 to 700 nm) over a wide range of intensities (sensitivity range 50 plx to 10 lx at 490 nm), and a video system consisting of a high-dynamic-range videocamera (1 in, Saticon), which is a Vidicon-type tube with Se-As-Tl light target photoconductor (Siemens, Karlsrule, Germany) linked to an image intensifier by high-transmission lenses. An objective focuses the luminescent signal on a photocathode in the image intensifier, which are then amplified. Another lens projects the image to the Saticon tube, and the final image is then processed. The samples are placed in a light-tight box to prevent interference by external light.

A very flexible approach to the problem of getting light around the optical path of the instrument is to use fiber optic light guides.[20] An optical fiber coupled light-emitting, diode-based absorbance detector with a reflective flow cell is described.[21] The optical fibers are used to carry light from the electronics/display unit to a reflective flow-through cell and back. It is well known that an optical fiber carries light with minimal loss because of a refractive index (RI) difference between the core and the cladding of the fibre. Thus, the cell can be located remotely from the electronics unit and the umbilical connection is not susceptible to electrical noise. The noise level of this detector with light-emitting diodes (LEDs) of different emission maxima were observed to be in the range 3 to 20 μAU, with a maximum short-term drift of 4 μAU/min after the initial warmup period. When the analyte absorbance is well matched with the source emission characteristics, the detector response is linear with concentration over at least two orders of magnitude. The utilization of fibre optics decreases the detection limit of CL sensors significantly.[22-28]

B. Flow Injection Techniques

Most of the CL sensors are of the flow-through type. Flow injection analysis (FIA) is most useful for optimizing the reaction conditions for CL emission, and it is an important tool for the quantitative analysis of real samples. CL signals in flow systems increases proportionally to the analyte concentration, appear as sharp peaks superimposed on a low constant blank signal, measured as viewed by the time window when the mixture of analyte and reagents pass through the detector cell.[29]

The geometry of the cell plays a very important role in the quality of the analytical data obtained using CL flow-through sensors.[6] Different types of cells are proposed as shown in Figure 1.

For CL biosensors, Martin and Nieman proposed the following cell (Figure 2).[30]

For the proposed cell three different variations on the placement of enzyme and $Ru(bpy)_3^{2+}$ layers were tested. In the *series* design (Figure 3a) the enzyme loaded film is adjacent to, but upstream from, the Nafion/$Ru(bpy)_3^{2+}$ film. In Figure 3b, the *stacked* approach places the layers on top of one another. The *opposite* approach places the two layers directly across the thin film channel from each other (Figure 3c). The advantage of the stacked and opposite approaches over the series is the ability to use both electrodes for CL detection. The advantage of opposite over stacked is a shorter preparation time (both layers prepared at the same time versus one after the other) and the potential ability to preserve the enzyme layer for reuse with a new CL sensor.

Most optical cells used for flow-through applications have got problems from the refractive index (RI). To reduce changes in the RI it is necessary to image a source on the exit window with focusing optics and to use a tapered cell construction. The utilization of a second wavelength as reference can also reduce the changes in RI. In a conventional Z-cell, the light path is colinear with the liquid flow path. The reflective cell design, on the contrary, the light path is orthogonal to the liquid flow path and the light reflected back almost along the same path, reducing RI sensitivity substantially.

One of the major problems for flow-through optical cells is the various degree of sensitivity to bubble or foreign particle entrapment problems. This can be solved by using the radial path across the flow conduit as the optical path. The entrapment problems are reduced for a linear flow path and a large exit bore.

A reflective cell that exhibits low RI susceptibility is described by Jambunathan et al. (Figure 4).[21] The cell itself is composed of a (inside dimensions 3 × 3 mm) square cross-section glass or quartz tubing T (Vitrocom, Mountain Lakes, NJ) that has been tapered as shown on the entrance side to an inner diameter of 0.7 mm to match with the conduit inner diameters used in the flow manifold. The smooth taper provides a smooth flow transition and good washout profiles without leading to added flow noise. O-rings on both sides provide the necessary liquid sealing. Into insert I two 1.5-mm core, jacketed technical-grade acrylic optical fibers (Edmund Scientific, Barrington, NJ) F1 and F2 are pushed throughput. The insert fits snug into an appropriately driller hole in the cell holder body, B. The fiber optic-bearing insert is pushed flush to address the cell face and then fixed in the place in the correct orientation with a retaining screw. On the opposite side of the cell, arrangements are made to reflect the launched light. In an initial design, a front surface concave mirror, C, was placed on the obverse face of the cell with a retaining nut, N. The simple expedient of silvering the outside of the cell body itself (Silvering Kit E-0060, Wilt Industries, Lake Pleasant, NY) provides the same results. In silvering, care should be taken to cover the entrance and the exit apertures of the cell to prevent the internal surface from being silvered. It was find

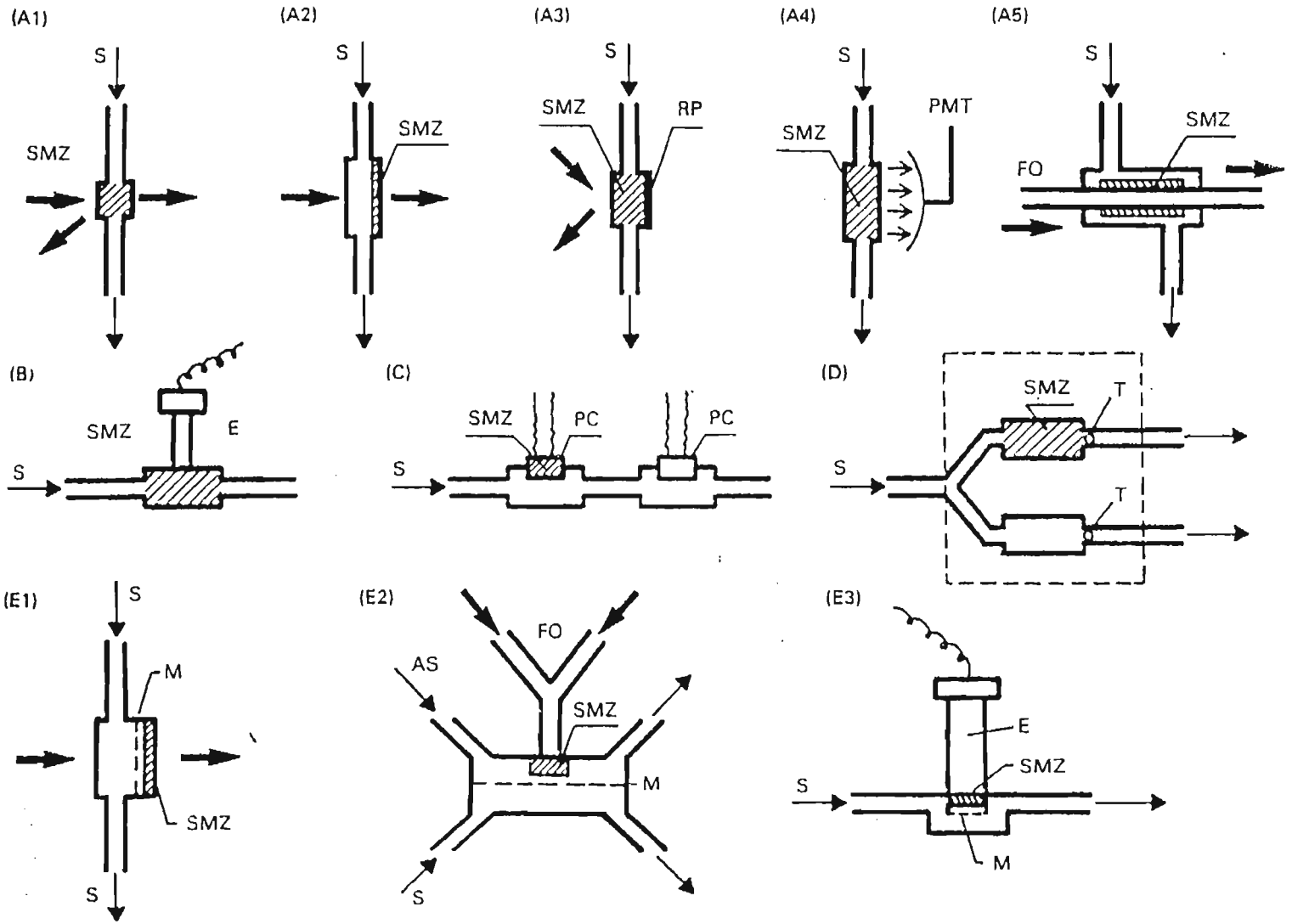

FIGURE 1. Examples of flow-through cell sensors in which a sensing (bio)chemical microzone (SMZ) (type A, B, C, and D) and a membrane (M) (type E) are integrated with an detection system.[6]

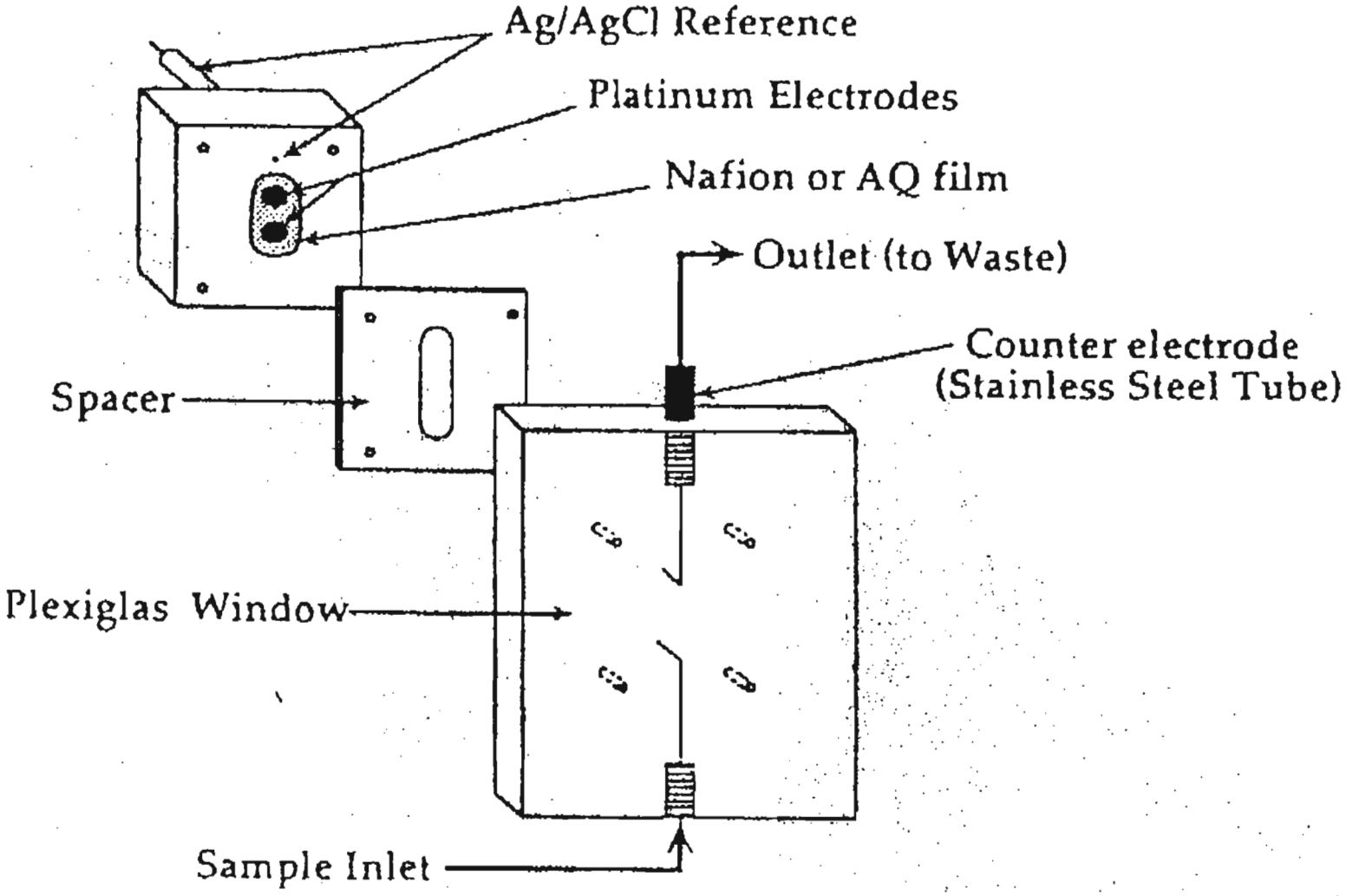

FIGURE 2. Expanded view of ECL flow cell.[30]

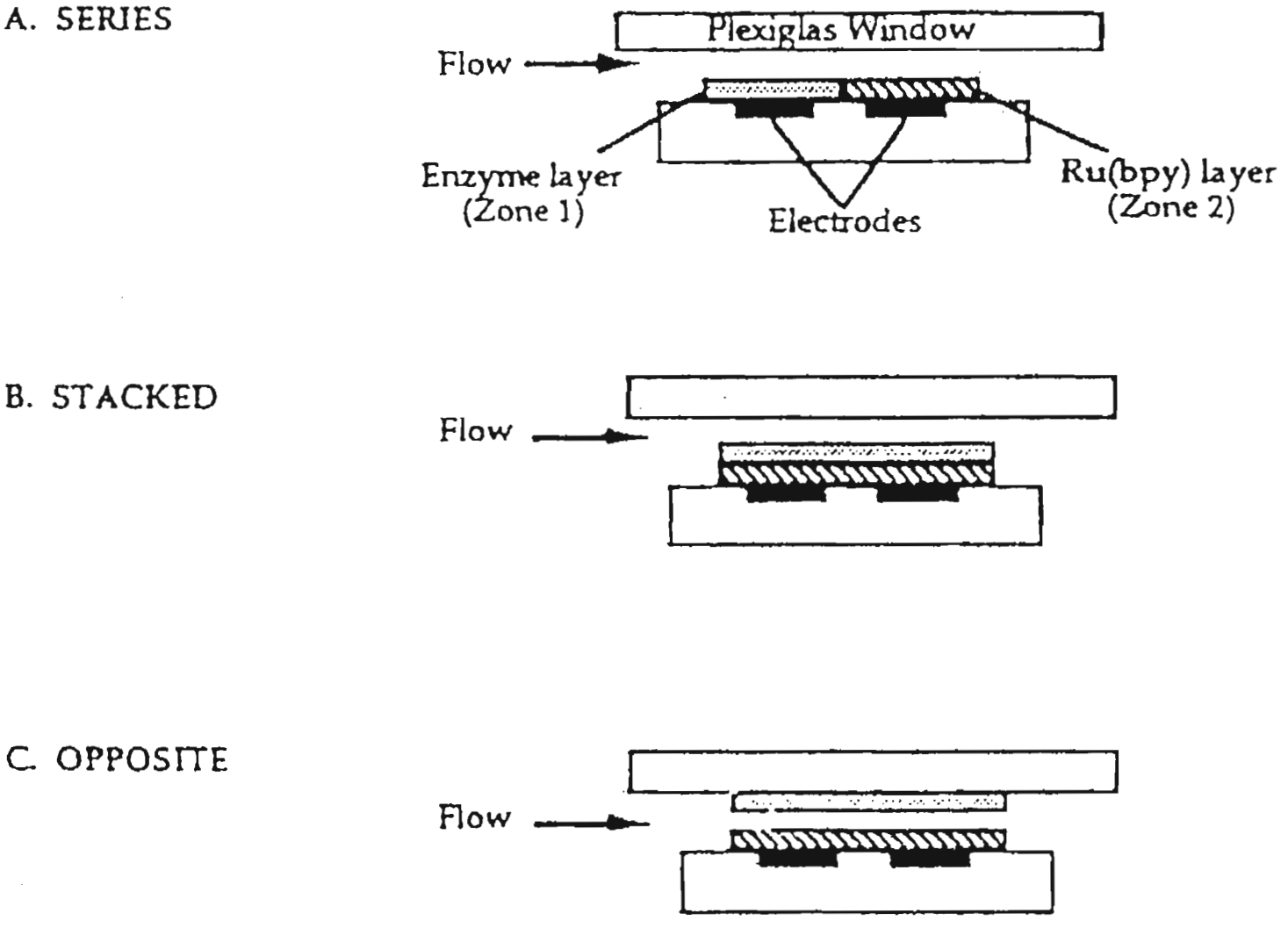

FIGURE 3. Three ECL biosensor designs for enzyme and $Ru(bpy)_3^{2+}$ layers.[30]

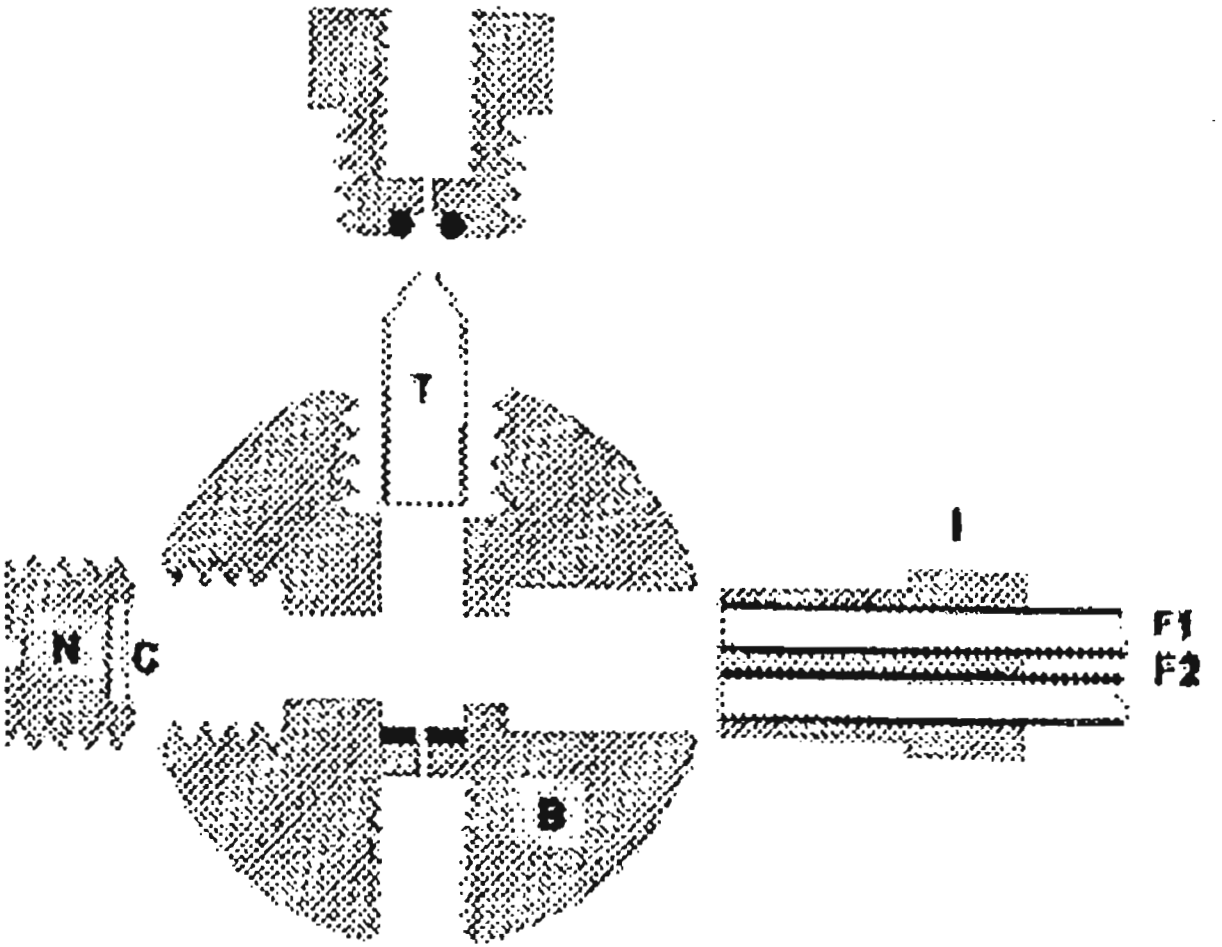

FIGURE 4. Cell design. T, rectangular cross section glass/quartz cell, put in cell body, B, and sealed by O-ring seals on either side. IN/OUT connections are 1/4-28 threaded. Insert carries fiber optics F1 and F2 that sit flush against the cell wall. Front surface concave mirror C is held by nut N on the observe side of the cell; alternatively, the cell is silvered on all but the face addressed by the optical fibers.[21]

expedient to silver the entire exterior of the cell and then remove the silver from the optically addressed face. The silver coating is protected by a clear acrylic spray coating.

A high importance in the decreasing of the RI has got the material used for cell construction. It was determined that the cell has to have a lower RI than the liquid. The water was considered as reference for the RI of the materials used in cell construction. The following construction materials were described: carbon disulfide (RI 1.63 for Na D-line) in glass tubes (RI typically 1.52)[31] or ethanol (RI 1.36) in a fluorinated ethylene-propylene (FEP) copolymer (RI 1.34) tube.[32]

The best results were obtained by the utilization of an amorphous fluoropolymer, Teflon AF, which has an RI less than that of water.[33] This material can be successfully used for liquid core waveguide cells (Figure 5).[34] The main advantage of the utilization of these cells is that the cell is behaving like an optical fiber or waveguide. The sensitivity of measurements increases significantly due to the fact the cell is glued to the detector and light source.

There are three basic types of flow-through sensors according to the location on the sensitive microzone where the reaction takes place and in the relationship with the detector (Figure 6).[6] Two of them (a and b) rely on the use of probes connected to the instrument; the sensitive microzone can be attached to the end of the probe (a) or incorporated into the flow cell (b). The third type of sensor involves a conventional instrument in which the sensitive microzone is incorporated into a special flow cell (c).

III. APPLICATIONS

A. Determination of Analytes in Air or Vapor

1. NO_2 Sensors

Three types of CL sensors are reported for the NO_2 assay. Heilmann et al.[35] proposed a lead phthalocyanine thin film sensor. The sensor chip consisted of a thin film layer (200 nm) prepared by vacuum sublimation of purified powder of lead phthalocianine and comb-like Au electrodes. The best sensitivities can be achieved by the utilization of the CL biosensors proposed by Spicer et al.:[36] the first one is based on the reduction of NO_2 to NO followed by the detection of NO by the CL produced from its reaction with O_3, while the second one is based on the detection of CL produced from the reaction of NO_2 with luminol solution. The working concentration range for the O_3 CL sensor (0 to 800 μg/l NO_2) is larger than the working concentration range obtained using the luminol CL sensor (0 to 50 μg/l). The main disadvantage of these types of CL sensors is non-selectivity over a lot of substances (e.g., nitrous acid).

2. O_2 Sensor

The system described for the O_2 assay[37] incorporates a solid-phase reagent across which the air under test is pumped, positioned below a photomultiplier tube for measurement of the resulting CL. Of the hydrogel or polymeric sorbents investigated, a fluoropolyol incorporating luminol, KOH, and iron (III) sulfate as catalyst gave the best results for the O_2 assay, having a detection limit of 2.4 mg/l.

3. Hydrazine Sensor

For the assay of hydrazine and its monomethyl and dimethyl derivatives, two types of experiments with CL sensors are proposed, according to *ex situ* and *in situ* types of analysis.[38] For the *ex situ* method, an additional device is necessary: the sample diluted in air was delivered at 1 ml/min by diffusion through a cellulose membrane to a chamber containing *tris*(2,2' bipyridyl)-

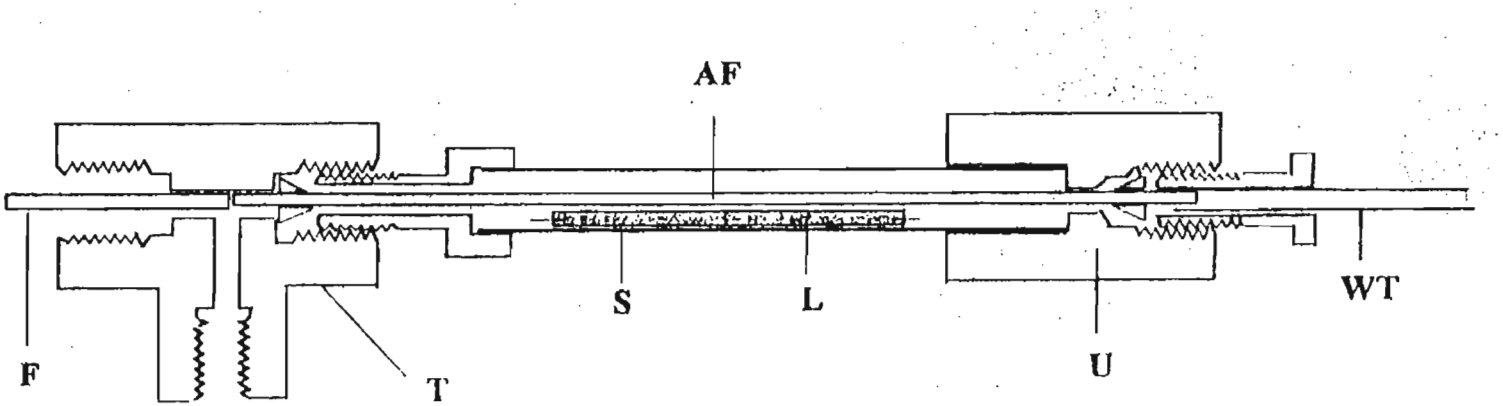

FIGURE 5. Typical arrangement for chemiluminescence and fluorescence detections. F, acrylate or silica optical fiber butted against LCW tube AF in the center of tee fitting T. The other end of AF is connected to waste tubing WT by compression fitting union U. A tubular shell S houses the tubing AF and the light source L.[34]

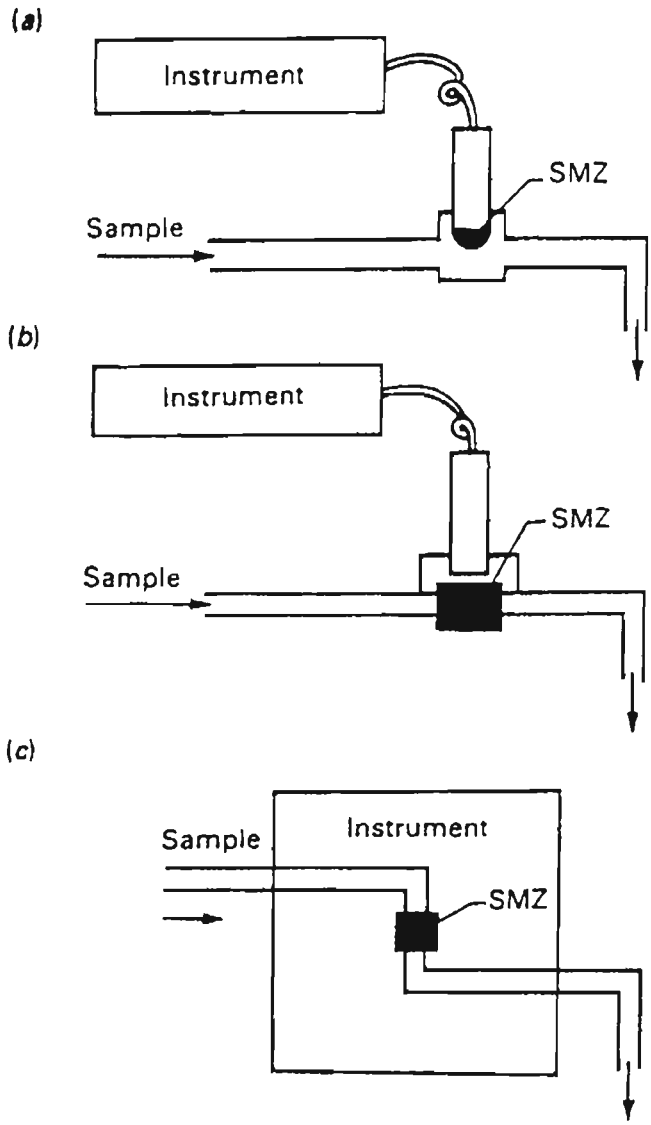

FIGURE 6. Generic types of flow-through (bio)chemical sensors.[6]

ruthenium complex in 0.1 mol/l phosphate buffer; the analytes were oxidized using a Pt working electrode. The detection limits are on ng/l concentration level for both *in situ* and *ex situ* analyses.

4. Sulfur-Containing Compounds Sensors

Meng et al.[8] described a CL sensor based on *tris*(2,2'-bipyridyl)ruthenium(II) permanganate for the SO_2 assay. SO_2 can be sampled if air is purged through a 0.1% triethanolamine absorbing solution. Furthermore, the slope of the calibration graph is constant for a given triethanolamine solution, and it was stepwise linear from 1×10^{-7} to 1.25×10^{-5} mol/l of SO_2 in the triethanolamine solution. The recovery of SO_2 in air samples is between 94.6 and 105.4%.

A high-speed sensor for the assay of dimethyl sulfide in the marine troposphere based on its CL reaction with F_2 is reported.[39] Sample air and F_2 in He were introduced at opposite ends of a reaction cell with a window at one end. The production of vibrationally excited HF and electronically

excited fluorohydrocarbon (FHC) was monitored with a photomultiplier tube. Dimethyl sulfide can be determined in the 0 to 1200 pptv (parts per trillion by volume) concentration range, with a 4 pptv detection limit.

5. Other

CL gas sensors are proposed for the discrimination and determination of constituents in mixed gases. Two types of CL gas sensors are proposed. They are based on α-Al_2O_3 supported on a ceramic substrate[40] or on an α-Al_2O_3 substrate,[41,42] respectively. The sensors were mounted in a chamber through which air/vapor flowed at a constant rate.

The detection limit is 1 ppm for acetone, ethanol, and butanol. Using the ceramic support, these components could be determined up to 1000 ppm concentration level.[40] For ethanol and butanol assay using as support α-Al_2O_3 the linear range covered only two magnitudes order.

B. Determination of Analytes in Liquids

1. Enzyme-Based Sensors

a. Amino Acids

A CL biosensor for amino acids was constructed from a transparent PTFE tube containing amino acid dehydrogenase, NADH oxidase, and peroxidase immobilized on tresylated hydrophilic vinyl polymer beads that was coiled spirally in front of a photomultiplier tube.[43] The sensor is free of interferences from proteins and NH_4^+. The reducing agents, for example, glutathione do intefere. The calibration graph was linear from 30 nmol/l to 5 µmol/l amino acid, with a detection limit of 10 nmol/l. The rate of samples was: 75 samples/day. The RSD values are less than 2.0%. The recovery of amino acids in plasma was 98 to 100%.

A packed bed flow microreactor containing alanine aminotransferase immobilized on sieved porous glass beads was combined with a CL detector.[44] To catalyze the indicator reaction between luminol and H_2O_2, Co(II) and immobilized peroxidase from *Arthromyces ramosus* (ARP) were used in a fiber optic cell. L-alanine was determined from cell cultivation media in the 2 to 500 µmol/l concentration range, with a limit of detection of 1 µmol/l using Co(II), and in the 5 to 800 µmol/l concentration range with a limit of detection of 2 µmol/l when ARP was used.

b. Choline

The detection of choline using a CL biosensor is based on the immobilization on a polymer[45] or nylon[46] of choline oxidase and fungal peroxidase. The calibration graphs were linear in 0.1 to 1 µmol/l concentration range with a limit of detection of 1 µmol/l.

c. Ethanol

Alcohol oxidase was used to generate H_2O_2 followed by its reaction with luminol in the presence of $K_3[Fe(CN)_6]$ as catalyst.[47] The luminescence was transmitted from the flow cell via optical fibers to the detector. Ethanol can be determined in the 3 to 750 µmol/l concentration range, with a detection limit of 3 µmol/l.

d. Glucose

Several types of CL biosensors are described for glucose assay. The first type was based on utilization of glucose oxidase for enzymatic reaction in coupling with luminol for CL reaction.[48,49] The working concentra-

tion range is on mmol/l magnitude order, with a detection limit of 1 μmol/l. The second type was based on the utilization of glucose dehydrogenase enzyme in coupling with *tris*(2,2'-bipyridyl)ruthenium (II) complex.[50] This sensor can be used on 10 to 2500 μmol/l concentration range. There are a lot of interfences such as NADH, oxalate, proline, and tripropylamine. However, gluconic acid and NAD^+ do not interfere.

e. L-Lactate

Enzyme-modified silica and graphite paste were used to construct the CL biosensor for L-lactate.[51] L-lactate oxidase was coupled with luminol/Na_2CO_3 (pH = 9.2) to generate the CL. The system is very sensitive and selective when it is used in clinical analysis.

f. NADH

A regenerable electrogenerated CL biosensor for NADH, based on dehydrogenase and *tris*(2,2'-bipyridyl) rythenium (II) complex immobilized on Eastman AQ 55S and Nafion cation-exchange polymer films is reported.[30] The working concentration range is 0.2 to 5 nmol/l. Blume et al.[52] proposed a bioluminescence biosensor for the assay of NADH. A bioactive layer was designed using a commercial preactivated polyamide membrane to which bacterial luciferase and oxidoreductase were covalently bound. The calibration graph was linear in the 10 pmol/l to 0.5 nmol/l concentration range.

g. H_2O_2

Horseradish peroxidase (HRP)-based biosensors are proposed for the assay of H_2O_2.[53,54] HRP was immobilized by microencapsulation in sol-gel crystals derived from tetramethyl orthosilicate.[54] H_2O_2 can be assayed in the 0.1 to 3 nmol/l concentration range, with a detection limit of 10^{-4} mol/l magnitude order.

h. Xanthine and Hypoxanthine

CL enzyme sensors for xanthine and hypoxanthine assay are developed by covalent immobilization of microbial peroxidase (from *Thromyces ramosus*) or xanthine oxidase, on preactivated nylon membranes.[55] CL was produced based on the luminol-H_2O_2 reaction. The detection limit was of 5 μmol/l.

2. Microbial Sensors

A CL microbial sensor is described for glucose assay.[56] For sensor construction, the microbe cells were impregnated on a chitosan gel that was applied to the pH-sensitive field-effect transistor (FET) surface. Glucose can be assayed using this sensor in 0.1 to 1 mmol/l concentration range.

3. Immunosensors

Aizawa et al.[57] described a genetically engineered molecular networks biosensing system in which the gene engineering was succesfully applied to develop biosensing material that includes an enzyme-labeled binding protein for enzyme immunosensing, a lipid-tagged single chain antibody for immunosensing and an environmentally responsive gene-bearing cell for environment sensing. Protein A was labeled with firefly luciferase according to the gene engineering to fuse proteins. A single chain antibody was genetically tagged with a lipid chain at a specific site by enzymatic modification of a preprotein in a transmembrane process within a bacterial cell. A recombinant plasmid bearing xyl R protein and firefly luciferase genes

with Ps promoter was transfected to *E. coli* to make the cell responsive to environmentally hazardous compounds such as xylene in emitting light.

A highly sensitive fiber optic immunosensor based on a metal-complex compound as chemiluminescence catalyst was used successfully in the tests of human serum albumin (HSA) as model protein, and anti-HSA antibodies raised in rabbits were immobilized on the optical fiber.[58] Competitive immunoassay was carried out by immersing the end of the optical fiber in a solution of HSA complexed with Fe(III) 2,9,16,23-tetrakis(chloro-carbonyl)phthalocyanine. The flow rates for phosphate buffer, H_2O_2, and luminol were 1.50, 0.25, and 0.25 ml/min, respectively. The HSA can be determined in the 0.1 to 100 mg/l concentration range, with a detection limit of 400 pg. The selectivity can be improved by utilization of a monoclonal antibody.

4. Other

a. Adrenaline

A flow-through CL sensor based on Mn(III)-tetrakis-(4-sulfonatophenyl)porphyrin immobilized on a dioctadecyl-dimethylammonium chloride bilayer membrane incorporated into a PVC-blended film was developed for the determination of adrenaline.[59] The calibration graph was linear from 3 μmol/l to 0.3 mmol/l of adrenaline solution. The sample assay rate was 40 samples/h, and the RSD (n = 10) for 50 μmol/l was found to be 1%.

b. Ascorbic Acid

Three sensors based on luminol and different cations immobilization on a resin are proposed for ascorbic acid assay, as follows: (1) D-201 type anion-exchange resin containing luminol and permanganate immobilized;[60] (2) D-201 × 7 anion-exchange resin was used for immobilization of luminol and 732 cation-exchange resin (Na form) was used for Fe(II) immobilization;[61] (3) amberlit A-27 anion-exchange resin containing immobilized luminol and potassium ferrycyanide.[62] Using these types of flow-through sensors, ascorbic acid can be assayed on: (1) 10 μg/l–4 mg/l; (2) 1 nmol/l–1 μmol/l, and (3) 0.01–0.8 μg/ml concentration ranges, with the following detection limits: (1) 5 μg/l; (2) 0.4 nmol/l; (3) 5.5 ng/ml, respectively, while the first proposed sensor is free of interferences. Cu(II), thiourea, uric acid, and vitamin B_1 seriously interfere with the third sensor.

c. Chlorine

The sensor proposed for chlorine assay[63] consisted on a Pyrex tube, packed with the uranine [fluoresceine disodium] complex immobilized on IRA-93 anion-exchange resin, and a photomultiplier tube placed close to the Pyrex tube. It was used for the monitoring of the concentration of free chlorine (as HClO) in tap water, up to 1 mmol/l, with a detection limit of 2 μmol/l. The coefficient of variation (n = 10) obtained for the free chlorine assay is 1.6%, for a concentration of 10 μmol/l. The main disadvantage is the short life time of the sensor.

d. Copper

The copper CL sensor[64] comprised an anion-exchange column having luminol and cyanide co-immobilized on the resin, while Cu was temporarily retained by electrochemical preconcentration on a Au electrode placed in an anodic stripping voltammetric cell. Injection of 0.1 mol/l NaOH through the column elutes the reagents, which then re-

acted with Cu stripped from the electrode to produce a CL signal. The response was linear in the 0.01 to 10 μg/l Cu(II) solution concentration range, with a detection limit of 8 ng/l. The RSD value at 40 ng/l concentration level was 7.4%, for the assay of Cu(II) in natural waters and human serum.

e. Ethanol

The CL sensors described for ethanol assay from water comprising a (α-Al_2O_3 layer that can be coated with a Pt thin film.[65] The limit of detection is on mg/l magnitude order. The CL reaction is based on ethanol oxidation.

f. Oxalate

An electrode based on *tris*(2,2'-bipyridyl)ruthenium (II) complex immobilized in a Nafion film is proposed for CL assay of oxalate.[66] The limit of detection is 1 μmol/l, and the working concentration range is over four orders of magnitude.

g. H_2O_2

The assay of H_2O_2 can be done using a CL sensor in the presence of luminol and Co(II)[67,68] or Cu(II)[67] ions. Using Co(II) and Cu(II) foils,[67] H_2O_2 can be determined in the 0.1 to 200 μmol/l and 5 to 200 μmol/l concentration range, respectively. To improve the selectivity, a gas dialysis cell was used. By immobilization of luminol and Co(II) on a strongly basic anion-exchange resin and a weakly acid cation-exchange resin, H_2O_2 can be determined in the 40 nmol/l to 10 μmol/l concentration range with a limit of detection of 12 nmol/l. The high sensitivity of the last system[68] made the assay of glucose in blood possible; the selectivity is improved by utilization of a packed bed reactor with immobilizatized glucose oxidase.

h. S^{2-}

The flow-through sensor proposed for S^{2-} assay[69] consists of a glass column packed with a homogeneous bed of resin with permanganate and another resin with riboflavin phosphate. CL was transduced to an electrical signal with a photomultiplier tube. S^{2-} can be assayed from beer and wine on 0.1 to 100 mg/l concentration range with a detection limit of 0.06 mg/l, and a RSD of 3.7% for 1.0 mg/l of sulfite.

i. $S_2O_8^{2-}$

A microring electrode is reported for $S_2O_8^{2-}$ assay.[70] A gold-coated fiber was polished to a flat surface, such that the gold formed a microring electrode around the optical fiber. *Tris*(2,2'-bipyridyl)ruthenium (II) was used as reagent for CL generation. The detection limit was 4 nmol/l.

j. Trichlorethylene

An optical-fiber CL sensor is reported for trichlorethylene assay.[71] The sensor consisted of a glass fiber bundle and a transducer consisting of three components: (1) a gas-permeable membrane to separate trichlorethylene from water, (2) H_2SO_4-$NaNO_3$ mixture as oxidizing agent, and (3) luminol solution. The assay of trichloroethylene can be done on 0.05 to 0.6 μg/ml concentration range with a detection limit of 0.03 μg/ml.

k. Uric Acid

For the assay of uric acid, a sensor based on $KMnO_4$-octylphenyl polyglycol ether is proposed.[72] Uric acid can be assayed directly, in urine in the 0.10 to 600 μg/ml concentration range with a detection limit of 55 ng/ml. The system is free of interferences.

IV. CONCLUSION

CL sensors are very important tools in analytical chemistry, due to their high sensitivity. The selectivity of these sensors was improved by utilization of an enzymatic or antigen-antibody reaction. It is essential in the future to improve the selectivity of CL reaction by the use of new reagents. However, they are also very sensitive to a variety of environmental factors such as temperature, solvent, ionic strength, pH, and other species present in the matrices.

The utilization of the fiber optic improved the quality of light detection. New types of flow-through cells are recommended for the CL sensor construction. The sensitivity and selectivity achieved so far make possible the utilization of CL sensors for the determination of inorganic and organic compounds from gaseous samples and solutions with good reproducibility and selectivity at low concentration level.

REFERENCES

1. Simpson JSA, Campbell AK, Ryall MET, Woodhead JS. *Nature A stable chemiluminescent-labelled antibody for immunological assays*. **1979** 279, 646-647.
2. Whitehead TP, Thorpe GHG, Carter TJN, Groucutt C, Kricka LJ. Nature *Enhanced luminescence procedure for sensitive determination of peroxidase-labelled conjugates in immunoassay*. **1983** 305, 158-159.
3. Bakker E. Anal. Chim. Acta *Selectivity comparison of neutral carrier-based ion-selective optical and potentiometric sensing schemes*. **1997** 350, 329-340.
4. Ouyang J, Baeyens WRG, Delanghe J, Van Der Weken G, De Keukeleire D, Calokerinos AC. Biomed.Chromatogr. *Flow-injection analysis of hydrochlorothiazide applying sensitised chemiluminescence detection: optimisation in view of narrow-bore HPLC*. **1998** 12, 162-163.
5. Zhang ZJ, Qin W. Fenxi Kexue Xubao *Fibre-optic chemical sensors based on chemiluminescence and bioluminescence*. **1997** 13, 72-77.
6. Valcárcel M, Luque de Castro M. Analyst *Flow-through (bio)chemical sensors*. **1993** 118, 593-600.
7. Ishii M, Yamada M. J. Flow Injection Anal. *Chemical sensors using flow injection techniques with chemiluminescence detection*. **1994** 11, 154-168.
8. Meng H, Wu F, He Z, Zeng Y. Talanta *Chemiluminescence determination of sulfite in sugar and sulfur dioxide in air using tris(2,2'-bipyridyl)ruthenium (II)-permanganate system*. **1999** 48, 571-577.
9. Huang Y, Zhang C, Zhang X, Zhang Z. Anal. Lett. *Cerium (IV)-based chemiluminescence analysis of analgin*. **1999** 32, 933-943.
10. Han HY, He ZK, Zeng YE. Fresenius J Anal. Chem. *A direct chemiluminescence method for the determination of nucleic acids using $Ru(phen)_3^{2+}$ -Ce(IV) system*. **1999** 364, 782-785.
11. Li Z, Li K, Tong S. Anal. Lett. *Study of the catalytic effect of copper (II) - protein complexes on luminol - H_2O_2 chemiluminescence reaction and its analytical application*. **1999** 32, 901-913.
12. Zhu Y, Wang K, Xu Y, He S. Huaxue Chuanganqi. *Multi-functional optical-fibre spectrophotometer*. **1993** 13, 44-47.
13. Stanley PE. J. Biolumin. Chemilumin. *Commercially available luminometers and imaging devices for low-light level measurements and kits and reagents utilizing bioluminescence or chemiluminescence: survey update*. **1997** 5. 12, 61-78.
14. Kricka LJ, Stanley PE. J. Biolumin. Chemilumin. *Bioluminescence and Chemiluminescence Literature*. **1998** 13, 157-184.
15. Kricka LJ. Anal. Chem. *Chemiluminescence and Bioluminescence*. **1999**. 71, 305R-308R.
16. Robards K, Worsfold PJ. Anal. Chim. Acta. *Analytical applications of liquid-phase chemiluminescence*. **1992** 266, 147-173.
17. Thorpe GHG, Kricka LJ, Moseley SB, Whitehead Th P. Clin. Chem. *Phenols as enhancers*

of the chemiluminescent horseradish peroxidase-luminol-hydrogen peroxidase reaction: application in luminescence-monitored enzyme immunoassay. **1985** 31, 1335-1341.

18. Michel Ph E, Fiaccabrino GC, de Rooij NF, Koudelka-Hep M. Anal. Chim. Acta *Integrated sensor for continuous flow electrochemiluminescent measurements of codeine with different rythenium complexes.* **1999** 392, 95-103.

19. Roda A, Pasini P, Musiani M, Girotti S, Beraldini M, Carrea G, Suozzi A. Anal. Chem. *Chemiluminescent low-light imaging of biospecific reactions on macro- and microsamples using a videocamera-based luminograph.* **1996** 68, 1073-1080.

20. Knowles A, Burgess C. *Practical Absorption Spectrometry,* Chapman and Hall Ltd., NY **1984.**

21. Jambunathan S, Dasgupta PK Wolcott DK, Marshall GD, Olson DC. Talanta *Optical fiber coupled light emitting diode based absorbance detector with a reflective flow cell.* **1999** 50, 481-490.

22. Spohn U, Preuschoff F, Blankenstein G, Janasek D, Kula MR, Hacker A. Anal. Chim. Acta *Chemiluminometric enzyme sensors for flow-injection analysis.* **1995** 303, 109-120.

23. Arnold MA, Zhou XG, Petsch RS. *Talanta Gas-sensing internal enzyme fibre optic biosensor for hydrogen peroxide.* **1994** 41, 783-787.

24. Preuschoff F, Spohn U, Weber E, Unverhau K, Mohr KH. Anal. Chim. Acta *Chemiluminometric L-lysine determination with immobilized lysine oxidase by flow injection analysis.* **1993** 280, 185-189.

25. Cattaneo MV, Luong JHT. Biotechnol.Bioeng *Monitoring glutamine in animal cell cultures using chemiluminescence fibre-optic biosensor.* **1993** 41, 659-665.

26. Starodub NF, Arenkov P Ya, Rachkov AE, Berezin VA. Sens. Actuators B Chem. *Optoimmunosensors for analysis of specific and non-specific classes of immunoglobulins.* **1992** 7, 371-375.

27. Zhang ZJ, Ma WB, Yang ML. Fenxi-Huaxue. *Fibre-optic biosensor based on chemiluminescence for the determination of uric acid.* **1992** 20, 1048-1051.

28. Ovchinnikov AN, Ogurstov VI, Trettnak W, Papkovsky DB. Anal. Lett. *Enzymatic flow-injection analysis of metabolites using new type of oxygen sensor membranes and phosphorescence phase measurements.* **1999** 32, 701-716.

29. Baeyens WRG, Schulman SG, Calokerinos AC, Zhao Y, Garcia-Campana AM, Nakashima K, de Keukeleire D. J. Pharm.Biomed. Anal. *Chemiluminescence - based detection: principles and analytical applications in flowing streams and in immunoassays.* **1998** 17, 941-953.

30. Stewart JE. Appl. Opt. *Optics of flow cells for liquid chromatography.* **1981** 20, 654-659.

31. Fujiwara K, Fuwa K. Anal. Chem. *Liquid-core optical-fibre total-reflection cell as a colorimetric detector flow-injection analysis.* **1985** 57, 1012-1016.

32. Tsunoda K, Nomura A, Yamada J, Nishi S. Appl. Spectrosc. *Use of poly(tetra-fluoroethylene-co-hexafluoro-propene) tubing as a waveguide capillary cell for liquid absorption spectrometry.* **1990** 44, 163-165.

33. DuPont Fluoroproducts. Teflon AF. Amorphous Fluoropolymers. H-16577-1, Wilmington, DE 19880-0711, December 1989.

34. Dasgupta PK, Genfa Z, Li J, Boring CB, Jambunathan S, Al-Horr R. Anal. Chem. *Luminescence detection with a liquid core waveguide.* **1999** 71, 1400-1407.

35. Heilmann A, Lantto V, Mueller M, Hamann C. Sens. Actuators B-Chem *Nitrogen dioxide monitoring as an air pollutant using lead phthalocyanine thin film sensors.* **1992** 7, 522-525.

36. Spicer CW, Kenny DV, Ward GF, Billick IH, Leslie NP. Air Waste *Evolution of NO_2 measurement methods for indoor air quality applications.* **1994** 44, 163-168.

37. Collins GE, Rose-Pehrsson SL. Anal.Chem. *Chemiluminescent chemical sensor for oxygen and nitrogen dioxide.* **1995** 67, 2224-2230.

38. Collins GE. Sens. Actuators B Chem. *Gas-phase chemical sensing using electrochemiluminescence.* **1996** 35, 202-206.

39. Hills AJ, Lenschow DH, Birks JW. Anal. Chem. *Dimethyl sulfite measurement by fluorine-induced chemiluminescence.* **1998** 70, 1735-1742.

40. Nakagawa M, Kawabata S, Nishiyama K, Utsunomiya K, Yamamoto I, Wada T, Yamashita Y, Yamashita N. Sens. Actuator B Chem. *Analytical detection system of mixed odour vapours using chemiluminescence-based sensor.* **1996** 34, 334-338.

41. Utsunomiya K, Nakagawa M, Sanari N, Kohota M, Tomiyama T, Yamamoto I, Wada T, Yamashita N, Yamashita Y. Sens. Actuator B Chem. *Continuous determination and doscrimination of mixed odour vapours by new chemiluminescence-based sensor system.* **1995** 25, 790-793.

42. Nakagawa MA. Sens. Actuator B Chem. *New chemiluminescence based sensor for discriminating and determining constituents in mixed gases.* **1995** 29, 94-100.

43. Kiba N, Tachibana M, Tani K, Miwa. Anal. Chim. Acta *Chemiluminometric branched chain amino-acids determination with immobilized enzymes by flow-injection analysis.* **1998** 375, 65-70.

44. Janasek D, Spohn U. Biosens. Bioelectron. *Chemiluminometric flow-injection analysis procedures for the enzymatic determination of L-alanine, a-ketoglutarate and L-glutamate.* **1999** 14, 123-129.

45. Zhou YK, Yuan XD, Hao QL, Ren S. Sens. Actuator B Chem *Development of a microbiosensor for the determination of choline and acetyilcholine.* **1993** 12, 37-40.

46. Lapp H, Spohn U, Janasek D. Anal. Lett. *An enzymic chemiluminescence optode for choline detection under flow-injection conditions.* **1996** 29, 1-17.

47. Xie X, Suleiman AA, Guilbault GG, Yang Z, Sun Z. Anal. Chim. Acta *Flow-injection determination of ethanol by fibre-optic chemiluminescence measurement.* **1992** 266, 325-329.

48. Suleiman AA, Villarta RL, Guilbault GG. Anal. Lett. *Flow-injection analysis of glucose by fibre-optic chemiluminescence measurement.* **1993** 26, 1493-1503.

49. Preston JP, Nieman TA. Anal. Chem. *An electrogenerated chemiluminescence probe and its application utilizing tris(2,2'-bipyridyl) ruthenium(II) and luminol chemiluminescence without flowing stream.* **1996** 68, 966-970.

50. Martin AF, Nieman TA. Anal. Chim. Acta *Glucose quantitation using an immobilized glucose dehydrogenase enzyme reactor and a tris(2,2'-bipyridyl) ruthenium(II) chemiluminescent sensor.* **1993** 281, 475-481.

51. Janasek D, Spohn U. Sens. Actuator B Chem. *An enzyme-modified chemiluminescence detector for hydrogen peroxide and oxidase substrates.* **1997** 39, 291-294.

52. Blum LJ, Gautier SM, Berger A, Michel PE, Coulet PR. Sens. Actuator B Chem. *Multicomponent organized bioactive layer for fibre-optic luminescent sensors.* **1995** 29, 1-9.

53. Arnold MA, Zhou XG, Petsch RS. Talanta *Gas-sensing internal enzyme fibre-optic biosensor for hydrogen peroxide.* **1994** 41, 783-787.

54. Diaz AN, Peinado MCR, Minguez MCT. Anal. Chim. Acta *Sol-gel horseradish peroxidase biosensor for hydrogen peroxide detection by chemiluminescence.* **1998** 363, 221-227.

55. Spohn U, Preuschoff F, Blankenstein G, Janasek D, Kula MR, Hacker A. Anal. Chim. Acta *Chemiluminometric enzyme sensors for flow-injection analysis.* **1995** 303, 109-120.

56. Reshetilov AN, Donova MV, Khomutov SM, Eliseeva TP. J. Anal. Chem. *Sensors based on field-effect transistors.* **1997** 52, 63-70.

57. Aizawa M, Yanagida Y, Haruyama T, Kobatake E. Sens. Actuator B Chem. *Genetically engineered molecular networks for biosensing systems.* **1998** 52, 204-211.

58. Hara T, Tsukagoshi K, Arai A, Imashiro Y. Bull. Chem. Soc. Jpn. *Highly sensitive fibre-optic immunosensor using a metal-complex compound as a chemiluminescence catalyst.* **1989** 62, 2844-2848.

59. Kuniyoshi A, Hatta K, Suzuki T, Masuda A, Yamada M. Anal. Lett. *Chemiluminescence sensor with manganese(III)-tetrakis(4-*

sulfonatophenyl)-porphyrin immobilized on dioctadecyl dimethylammonium chloride bilayer membranes incorporated into PVC film. **1996** 29, 673-685.

60. Wang FC, Qin W, Zhang ZJ. Fenxi Huaxue. *Flow-injection chemiluminescence sensor for the determination of ascorbic acid.* **1997** 25, 1255-1258.

61. Qin W, Zhang ZJ, Chen HH. Fresenius J. Anal. Chem. *Highly sensitive chemiluminescence flow sensor for ascorbic acid.* **1997** 358, 861-863.

62. Zhang ZJ, Qin W. Talanta *Chemiluminescence flow sensor for the determination of ascorbic acid with immobilized reagents.* **1996** 43, 119-124.

63. Nakagama T, Yamada M, Hobo T. Anal. Chim. Acta *Chemiluminescence sensor with uranine [fluorescein disodium] immobilized on an anion-exchange resin for monitoring free chlorine in tap-water.* **1990** 231, 7-12.

64. Qin W, Zhang ZJ, Liu HJ. Anal. Chem. *Chemiluminescence flow-through sensor for copper based on an anodic-stripping voltammetric flow cell and an ion-exchange column with immobilized reagents.* **1998** 70, 3579-3584.

65. Nakagawa M, Yamamoto I, Yamashita N. Anal. Sci. *Detection of organic molecules dissolved in water using a a-aluminium oxide chemiluminescence-based sensor.* **1998** 14, 209-214.

66. Downey TM, Nieman TA. Anal. Chem. *Chemiluminescence detection using regenerable tris-(2,2'-bipyridyl) ruthenium (II) immobilized in Nafion.* **1992** 64, 261-268.

67. Janasek D, Spohn U, Beckmann D. Sens. Actuator B Chem. *Novel chemiluminometric H_2O_2 sensors for the selective flow injection analysis.* **1998** 51, 107-113.

68. Qin W, Zhang ZJ, Li BX, Liu SN. Anal. Chim. Acta. *Chemiluminescence flow-sensing system for hydrogen peroxide with immobilized reagents.* **1998** 372, 357-363.

69. Qin W, Zhang ZJ, Zhang CJ. Anal. Chim. Acta. *Reagentless chemiluminescence flow sensor for sulfite.* **1998** 361, 201-203.

70. Kuhn LS, Weber A, Weber SG. Anal. Chem. *Micro-ring electrode-optical waveguide: electrochemical characterization and application to electrogenerated chemiluminescence.* **1990** 62, 1631-1636.

71. Bansho K, Tao H, Imagawa T, Miyazaki A. Bunseki Kagaku. *Preparation of optica-fibre trichloroethylene sensor for water with chemiluminescence detection after oxidative decomposition.* **1993** 42, 55-60.

72. Li Z, Feng ML, Lu JR. Mikrochem. J. *$KMnO_4$-octylphenyl polyglycol, ether chemiluminescence system for flow injection analysis of uric acid in urine.* **1998** 59, 278-283.