CHAPTER I

INTRODUCTION

Analytical Chemistry has become an invaluable tool in fields ranging from process control and environmental monitoring to the life sciences including pharmaceutical and clinical diagnostics. With rapidly evolving frontiers in genomics and proteomics, there is a vast demand for a higher throughput of analytical information. Demands on the throughput can concern the number of samples, the number of assays per sample, and time and budget. Miniaturized analytical systems can present a solution to these demands.

1.1 Miniaturized Analytical Systems

1.1.1 Concept [1-2]

For analytical methodologies, development and validation include optimization of some critical analytical parameters (e.g., accuracy, sensitivity, reproducibility, simplicity, cost effectiveness, flexibility and speed). However, other aspects concerning operator safety and environmental impact of analytical methods are not commonly considered. Because of that, a paradoxical situation emerged during the 1990s, due to the side effects of analytical methodologies developed to analyze different kinds of sample, including environmental samples that generate a large amount of chemical waste, resulting in a great environmental and human impact.

In some circumstances, the chemicals employed for analysis were even more toxic than the species being determined.

With this background, Green Analytical Chemistry (GAC) started as a search for practical alternatives to the off-line treatment of wastes and residues in order to replace polluting methodologies with clean ones.

Concerning the measurement step, greener analytical procedures are inherent to automated flow-based methodologies, due to their capability of reducing reagent and solvent consumption and also to the possibility of incorporating decontamination of wastes on-line. The development of flow-analysis-based techniques seems a search to minimize reagent consumption.

Sequential-injection analysis (SIA) is a robust alternative to classical flow injection (FI) that allows implementation of different flow methodologies without modification of the manifold. The main advantages of SIA over FIA are the dramatic reduction in the amounts of solvent and reagents consumed simplicity and reduction of wastes.

All current procedures could become environmentally friendly by reducing the amounts of reagents consumed and it can easily be achieved by downscaling the manifold components and arranging them in a single device. This concept is known as the micro-total analytical system (μ-TAS), which involves arranging all steps of sample processing in a single device of a few square centimeters.

To overcome these limitations, miniaturized versions, or micro (μ-TAS), were proposed. In μ-TAS, analytical components are integrated on planar chips. Feature sizes are typically in the micrometer range and high feature densities can be achieved. This was made possible through micromachining techniques developed in

the microelectronics industry. While the advantages of μ-TAS are gained through miniaturization and integration of the analytical components, peripheral equipment (e.g. for control and data acquisition) can be large. However, portable μ-TAS devices with compact peripheral equipment have a much wider application field. For instance, point-of-care (POC) diagnostic, in-the-field environmental monitoring and chemical/biological warfare detection become possible. Given the high degree of complexity that can be obtained, μ-TAS are often dubbed "Lab-on-chip", reflecting the potential of future systems to integrate entire chemical/biological laboratory procedures onto microchips.

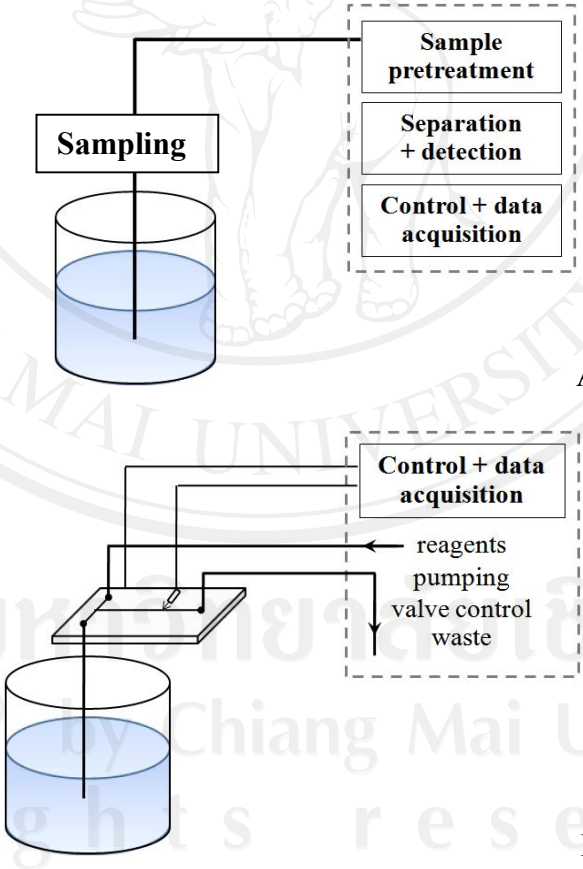


Figure 1.1 Different analytical systems: (A) Total chemical analysis system (TAS); (B) Miniaturized micro total chemical analysis system (μ-TAS) [2]

1.1.2 Theory of miniaturization

Combining the small size of detection systems with the analytical power of TAS, μ-TAS offer a number of distinct advantages. Changes of fundamental device characteristics as a function of miniaturization, expressed in terms of a typical device length *d*, are summarized in Table 1.1.

Table 1.1 Device characteristics for different typical length *d* values based on simple mitrotiter plate format [3]

Typical length	***1 mm***	***100 μm***	***10 μm***
Volume	10^{-6} L	10^{-9} L	10^{-12} L
No. of molecules for 1 μmol L^{-1}	6×10^{11}	6×10^{8}	6×10^{5}
Diffusion time	15 min	10 s	100 ms
Unit density	25 devices/cm^2	2500 devices/cm^2	2.5×10^{5} devices/cm^2
Information density	1.5 values/min/cm^2	250 values/ min/cm^2	2.5×10^{6} values/min/ cm^2

At the same time as the concept of μ-TAS was proposed, theoretical considerations in terms of similarity and proportionality were shaped. One approach employed dimensionless parameters to consider similarity; the other used the characteristic length of known systems versus scaled-down systems to consider proportionality. Dimensionless parameters can be used to correlate in an easy way experimental results when a great deal of variables are involved and are defined in terms of parameters that are assumed to be constant through the whole system under study. The Reynolds number, for example, used to characterize laminar and turbulent

flow regimes, is one of the parameters well known in fluidics. Using these parameters, the extrapolation of results obtained for one system to another is possible. It is less well known, however, that dimensionless parameters can be used for plate number and retention time in separation-based systems by expressing these variables in the constant factors of internal diameter, volume, and diameter.

On the other hand, the approach that considers proportionality provides helpful information related to the behavior of a simple flow system when miniaturized. If miniaturization is assumed as a downscaling process in three dimensions, represented by atypical length (d), the behavior of the physical variables of interest is predictable. For example, two systems can be distinguished, a time-constant system and a diffusion-controlled system. In the former, the time scale is the same in both large and miniaturized systems and variables such as analysis time and transport time stay unaffected, while others such as linear and volume flow rate decrease. Meanwhile, in the diffusion-controlled system, the time is regarded as a surface and proportional to d^2. Hence, this system is in accordance with the band-broadening theory in chromatography and electrophoresis, which means that diffusion processes such as heat diffusion, hydrodynamic diffusion, and molecular diffusion behave the same in both systems. In other words, if a system is downscaled by 1/10, the time variables decrease by a factor of 1/100, pressure increases by a factor of 100, and voltage requirements remain constant, but the essential chemistry and separation behavior retain the same quality [4].

1.2 Lab-on-a-chip technology [5]

The area of micro total analysis systems, also called “lab on a chip”, or miniaturized analysis systems, is growing rapidly. Lab-on-a-chip projects around the world are concentrating on miniaturizing laboratory trials to microscopic dimensions. The Lab-on-a-chip technologies currently used are still in their beginning, since it is still a relative new area of interest. The systems that are being developed are not only silicon based, as has been the trend before, but rather many scientists are looking at the possibilities of using polymer materials, both to make the fabrication cheaper, but also to have a wider range of possible uses. Materials such as thermoplasts, can be reused and molded in microscale shapes. Materials such as SU-8, has the advantage of being highly chemically stable and is a frequently used material, in the semiconductor industry as a photoresist. Another material often used, is PMMA, or plexiglass, which has the advantage of being cutable by laser, which makes it possible to make rough system of channels in few seconds. It can also easily be mechanically processed. The fabrication of labchips is mostly done by methods similar to the ones used in silicon fabrication, since this is an already highly developed process for very accurate processing.

1.2.1 Microfabrication

Most of the microfabrication technologies employed for miniaturized analytical systems was originally developed in the microelectronics industry. An overview of a standard fabrication process is given in Figure 1.2. The process comprises photopatterning of a protective layer, etching of the underlying substrate, and bonding to a cover plate to form enclosed microchannels.

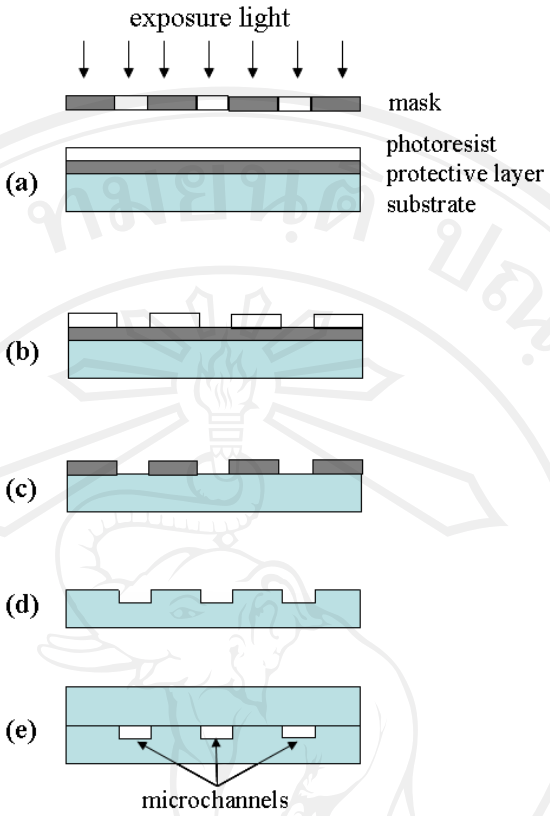


Figure 1.2 Microfabrication process for the production of microchannels; (a) Photolithographic patterning of photoresist, (b) Photoresist development, (c) Protective layer etching, (d) Substrate etching and (e) Bonding to cover plate [6]

This intention is to provide an introduction and reference source for readers new to microfabrication while giving more experienced readers a snapshot of processes that are most readily being applied in microfluidics.

1.2.1.1 Substrates

Factors to consider when choosing a substrate include temperature limits, chemical resistance, mechanical strength (bonding and substrate handling are often required) and optical properties. Interactions between test fluids and the substrate are also important as chip dissolution can change the properties of a fluid we are analyzing or manipulating. There are many substrates for a microfluidic chip such as silicone, glass (quartz and borofloat glass wafers) and plastics. As it was necessary to work with plastics polymers, so we are going to considerer are related to plastic.

The typical plastic materials for substrates include polyethylene terephthalate (PET), polymethyl methacrylate (PMMA), and polyimide. Plastic substrates usually come in large flat sheets, but they can easily be formed into workable sizes using a saw or laser cutter [7]. PMMA is probably the most common plastic substrate encountered in microfluidics and is classified as a thermoplastic and transparent plastic. It is commonly called acrylic glass or plexiglass. It is viewed as a potential substitute for glass and silicon-glass substrates for fabricating microfluidics, although it has higher optical absorption and more natural fluorescence for most wavelengths in and around the visible range. PMMA, like other plastics, must be processed at lower temperatures than semiconductor or glass wafers. Many solvents, like acetone, used for cleaning and photoresist removal are incompatible with PMMA.

1.2.1.2 Fabrication methods

A variety of methods exist for the fabrication of microfluidic devices, including wet etching, conventional machining, photolithography, hot embossing, and laser ablation. The process of removing material from a solid (or occasionally liquid)

surface by irradiating with a laser beam is laser ablation. It involves the use of a high-powered pulsed laser to remove material from a sheet of thermoplastic. Ablation using radiations of various wavelengths (IR, visible, UV, or x-ray) has been employed to fabricate plastic chips.

In addition to pulse energy, the depth of the ablated channels is also dependent upon the pulse rate and the absorption characteristics of the substrate. The minimal width of the ablated channel is determined by the focusing optics used to control the beam shape. CO_2 lasers, with wavelengths in the infrared region (10.6 μm), are also used for microfabrication, typically with PMMA or PET [8].

Direct laser patterning is a way to structure photosensitive materials in three dimensions [9]. Laser engraving is a practice that uses lasers to engrave or mark an object. A computer system is often used to control the movements of the laser beam and very precise engravings can be achieved at high rates on materials such as plastics and metals. Because light is utilized, laser engraving is a non-contact engraving method. In most systems, the work piece is stationary and the laser moves around in the *X* and *Y* directions drawing vectors (Figure 1.3).

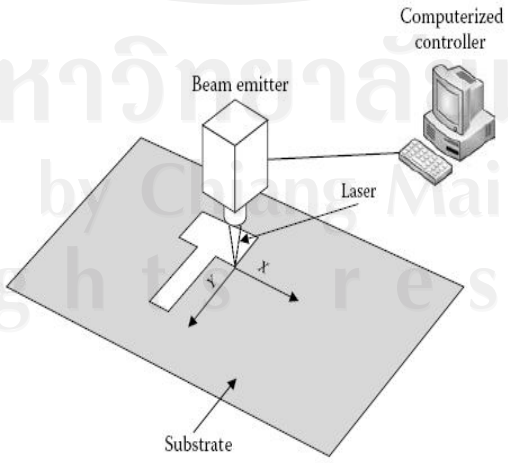


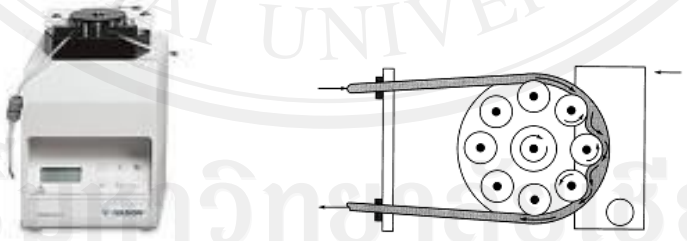
Figure 1.3 Laser engraving on a flat sheet

1.3 Microfluidic-Based Instrumentation

The instrumentation used for microfluidic integration of analytical control is very simple, and the microfluidic analyser depends on the particular requirements of the application used. The major components of a microfluidic system are as follows:

1.3.1 Moving and transport fluids on microchips

In general, on-chip pumping is either externally generated by traditional macroscale pumps or is integrated into microchips in the form of microfabricated components or by taking advantage of innate phenomena (e.g., surface tension) [10]. A peristaltic pump was used for moving solution in a microfluidic system. The peristaltic pump consists of a motor-driven wheel with peripherally placed rollers and a compression cam (or band) which is squeezed against the rollers. One or several pump tubes are affixed so that they rest on a minimum of the rollers at all times (as shown in Figure 1.4).



(a) **(b)**

Figure 1.4 Pump tubing and manifold connection tubes: (a) a peristaltic pump with two-channel head and (b) a flexible tube placed between the rotating head and a fixed piece. The rollers are squeezed onto the tubing [10]

Normally, the transport system consists of small-bore tubes such as PTFE tubing having 0.35 - 1.0 mm I.D. The other common pump used in microfluidic-based system, is a syringe pump, while the connectors used in a microfluidic system serve the purpose of joining the tubes to one another and to the other parts of the system.

A syringe pump is a small infusion pump (some include infuse and withdraw capability), used to gradually administer small amounts of fluid (with or without medication) to a patient or for use in chemical and biomedical research. Syringe pumps are also useful in microfluidic applications, such as microreactor design and testing, and also in chemistry for slow incorporation of a fixed volume of fluid into a solution (Figure 1.5).

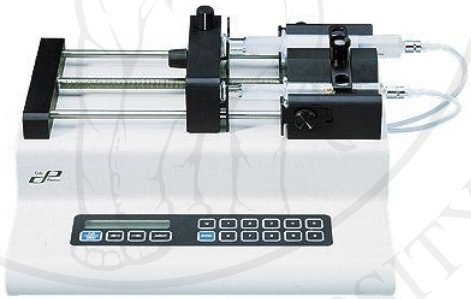


Figure 1.5 A Dual-syringe pump

1.3.2 Valving

The injectors employed in a microfluidic system are similar in kind to those used in HPLC and FI. For a successful analysis, it is vital that the sample solution is injected rapidly as a pulse or plug of liquid; in addition, the injections must not disturb the flow of the carrier stream. The earliest injection system employed in a microfluidic system was as simple as a syringe and hypodermic needle. Currently, the injection

systems most frequently used are the rotary valve, proportional injector and multi-injection system.

1.3.3 Detection strategies

The detection system is the sensing part of a microfluidic manifold, which allows continuous monitoring of a given property of the sample or its reaction product, and provides qualitative and quantitative information of the analyte. Many different types of detectors have been employed in a microfluidic system. Any detector that can be equipped with a microfluidic device can be adopted for use in a system. A variety of detection systems have been used in a microfluidic system. These include the UV-visible spectrophotometer [11], fluorimeter [12], various electrochemical [13] and chemiluminescence (CL) detectors [14]. In this thesis, only CL has been used as a detector in a microfluidic system, and it will be discussed in more detail later.

1.3.4 Data processing unit

In its simplest form, a microfluidic system may be manually operated, with the detector connected to a chart recorder for data acquisition and display. More advanced and commercial systems may be fully or partially automated, utilizing an on-board microprocessor system for timing of analyzer operations (i.e., sampling and injection), signal display, calibration and calculation of the results. More commonly, however, this control is achieved using a portable computer connected to a suitable analogue or digital converter, and a control/ data acquisition software [15].

1.4 Chemiluminescence

Chemiluminescence (CL) is currently an attractive detection technique because of its high sensitivity, low detection limit, selective reactions and wide dynamic range achievable with relatively simple equipment, especially in conjunction with a flow injection system. As CL is defined as the production of light arising from a chemical reaction, the only important equipment required is a sensitive photomultiplier tube [16] which makes the detection system simple and easy for fabricate in a small scale level.

1.4.1 Principle [17-18]

Chemiluminescence is the generation of electromagnetic (ultraviolet, visible, or near-infrared) radiation as light by the release of energy from a chemical reaction. While the light can, in principle, be emitted in the ultraviolet, visible or infrared region, those emitting visible light are the most common. They are also the most interesting and useful. Luminescent reactions can be grouped into three types:

(a) Chemical reactions using synthetic compounds and usually involving a highly oxidized species such as a peroxide are commonly termed chemiluminescent reactions.

(b) Light-emitting reactions arising from a living organism, such as the firefly or jellyfish, are commonly termed bioluminescent reactions.

(c) Light-emitting reactions which take place by the use of electrical current are designated electrochemiluminescent reactions.

The process by which CL is generated is the same as that for photoluminescence (e.g., fluorescence and phosphorescence), except that the former does not require light excitation source is not required.

1.4.2 The chemiluminescent reaction

There are many known chemiluminescent reactions to choose from in designing a detection scheme. However, only a fraction of the known reactions have found their way into high volume phamaceutical diagnostic testing methods. Several factors influence whether a particular chemiluminescent technology is suitable for use in automated immunoassays. For a chemical reaction to be suitable for CL detection, it should meet three essential requirements:

(a) The reaction molecule (C) should be capable of receiving the energy released from the reaction to form excited product (C*), and the efficiency of this process should be sufficiently high.

(b) The excited product (C*) should be capable of luminescing under the condition of the reaction; the intensity of the radiation should be sufficiently high. Alternatively, a suitable acceptor molecule, F, capable of accepting energy should be available for chemi-excited and subsequently emission of radiation.

(c) The energy required for excitation must be supplied by the reaction in one step, if possible. In a multi-step reaction, the necessary energy must be released in a single step since the excitation step should occur instantaneously.

The limiting factor for the occurrence of CL is that the energy required for luminescence in the visible region lies between 44 and 71 kcal mol^{-1}. Therefore,

a minimum requirement for CL is that the reaction produces 44 kcal mol^{-1} of energy. A variety of organic compounds meet this requirement and in some instances, their chemiluminogenic properties have been thoroughly studied during redox reactions. CL reactions can occur very rapidly (<1 s) or can be long lasting (>1 day), the duration being influenced by a wide range of the reaction condition. This presents a challenge to the development of an instrument for CL monitoring.

1.4.3 Measurement of chemiluminescence

Measuring the CL light produced from the reaction can be done in two ways: in static solutions or in a continuously flowing stream. In the static mode, only a portion of the chemiluminescence agent and the analyte are injected via syringe. The mixing is performed in a cuvette, which is accommodated in a luminometer chamber facing the photomultiple tube (PMT) window. The height or the peak area of the CL signal (Figure 1.6) is proportional to the analyte concentration and is used for quantitation of the analyte [19].

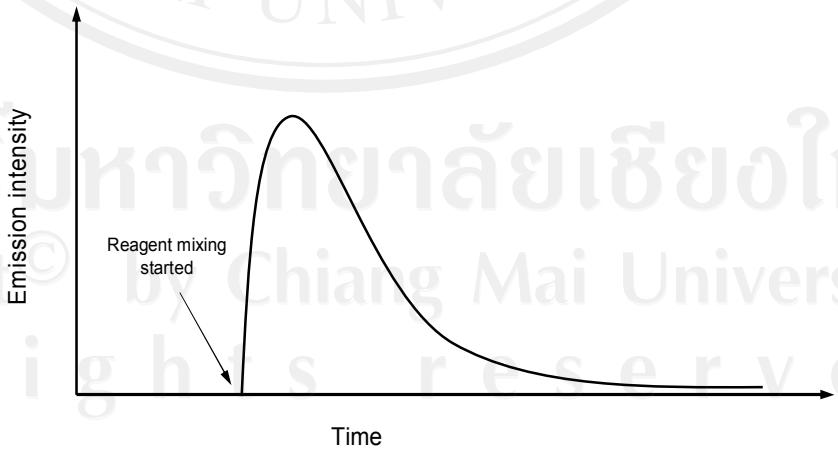


Figure 1.6 Typical chemiluminescence intensity signal obtained from static mode CL detection [19]

In the flowing systems, the chemiluminescent reagent and the analyte are continuously circulated through separate channels prior to merging into a single stream. Figure 1.7 illustrates a basic CL-flowing system setup, where the chemiluminescent reaction takes place upon merging of the reagent stream with the sample carrier stream. Satisfactory mixing is achieved effectively at a T-piece, but Y-junctions are equally efficient for this purpose.

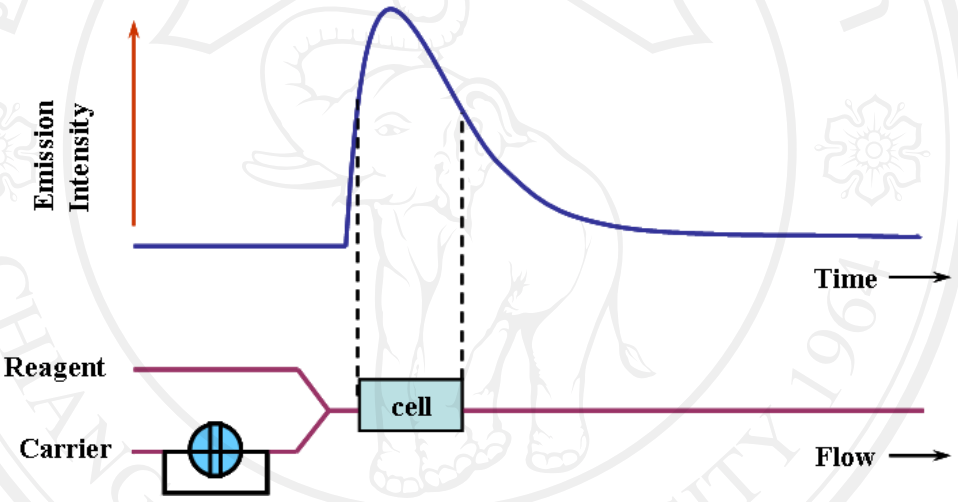


Figure 1.7 Position of observation window relative to the emission-time curve for flow through chemiluminescence mensurement

The application of chemiluminescence detection incorporated into a microfluidic system, which was reviewed recently by G.S. Fiorini and D.T. Chiuin in 2005 [20], has been successfully applied and proved to be beneficial for the quantitative determination of specific analytes in real sample matrices such as in pharmaceutical, environmental, animal feeds, and biomedical analyses.

1.5 Nitrofurans

The most common nitrofurans are furazolidone (FZD), furaltadone (FTD), nitrofurazone (NFZ), and nitrofurantoin (NFT) which has been banned in the European Union (Annex IV of Regulation 2377/90/EEC) due to their toxicological influence on the health of consumers (carcinogenicity and mutagenicity) of food from animal origin [21-22]. These antibiotics are also employed to treat bees infected with bacterial diseases, however, residues of these compounds were found in honey.

Drugs belonging to the group of 5-nitrofuran derivatives are well known and widely used due to their antimicrobial activity. Their mode of action is uncertain but appears to depend on the formation of reactive intermediates by reduction, which inactivate or alter bacterial ribosomal proteins and other macromolecules. Bacteria reduce nitrofuran drugs more rapidly than do mammalian cells, and this is thought to account for the selective antimicrobial activity of these compounds [23].

They were widely used as feed additives in food-producing animals like poultry, swine, cultured fish and shrimps, for treatment and prevention of various gastrointestinal infections caused by bacteria or protozoa and as growth promoters [24]. A great advantage of these compounds in comparison with other antimicrobial agents, is the slowly development and only to a limited extent of the *in vivo* bacterial resistance [25]. The most common nitrofurans are FZD, NFT and NFZ. These compounds are rapidly metabolized *in vivo*, leading to a significant decrease of their parent compounds levels in plasma. The elimination half-lives of nitrofurans are very short, and some hours after administration it is almost impossible to detect any residues of parent compounds in edible tissues. A concomitant accumulation of their protein-bound residues is observed, and their detection is possible over large periods

of time. Based on the evidence of carcinogenic and genotoxic effects of these bound metabolites, European Union (EU) has forbidden, for more than a decade, the use of nitrofuran drugs in food producing animals[21].

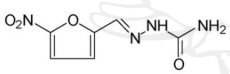
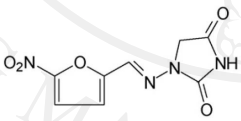
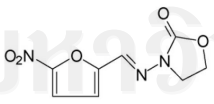
Nitrofuran compounds were usually administered to animals by means of medicated feeds or at drinking water. Concentrations in feeds ranging from 8 to 400 mg kg^{-1} were considered appropriate depending of the intended use.

This means that low concentrations of these compounds can be the source of nitrofuran residue metabolites in meat and other edible products obtained from animals consuming the contaminated feed. So, animal feeding stuffs must be analyzed with analytical procedures capable of measuring very low concentrations of nitrofurans to assure its “fit-for-purpose”.

Nitrofuran derivatives are highly effective chemotherapeutic drugs, well known as antibacterial agents since Dodd and Stillman's studies. Some of them are widely used to fight common infections of humans and animals or characteristic infections of domestic animals or poultry. They are a coccidiostat used in poultry and swine feed and papers on its determination in pharmaceuticals, biological fluids, animal substrates and feeds by different techniques. The aim of this research was to design, construct, to develop a methodology for screening and confirmation the presence of FZD, NFT and NFZ at low concentrations in animal feed, in order to satisfy the above referred needs on feed control. In addition, quantitative analysis of nitrofurans in pharmaceuticals necessary for monitoring the quality control in platform of these drugs and assures these drugs are set of regulations in specified gradient. The application of the method is extended to the assay of nitrofurans in animal feeds will be also included [21].

This research describes the utility of microfluidic and chemiluninescence for determination of these compounds in their pure forms, pharmaceutical formulations and animal feeds. In addition, the purposed method can be used as alternative method in green analytical chemistry for determination of these compounds in pharmaceutical preparation and animal feeds with simple and economical instrument. The three compounds under study in this paper as shows in Table 1.2.

Table 1.2 List of the studied compounds

Compounds	Structure	Mol. wt.	Medicinal uses	Manufacturer
Nitrofurazone		198.14	Local antibacterial veterinary use : surface bacterial in fections of the skin	Aldrich, Chem. Co.
Nitrofurantoin		238.1 6	Urinary antiseptic Veterinary use : for canine Tracheobronchitis	Aldrich, Chem. Co.
Furazolidone		225.16	Antimicrobial, veterinary use : protozoal diseases	Sigma Chem. Co.

1.5.1 Nitrofurazone

Nitrofurazone (NFZ) is bactericidal against most bacteria commonly causing surface infections. It is used as a local application for wounds, burns, ulcers, skin infections and for the preparation of surfaces before skin grafting. Various dosage forms are available as ointment or topical solution [26]. Nitrofurazone is also used as a feed additive in food-producing animals to control intestinal bacterial infections and coccidiosis. However, in the European Union, the use of nitrofurans in food producing animals is prohibited [27].

1.5.2 Nitrofurantoin

Nitrofurantoin (NFT) is bactericidal to many grampositive and gram-negative pathogens. It is readily absorbed from the gastrointestinal tract and is used in the treatment of urinary tract infections. It is used to treat urinary infections and can be administered orally or parenterally. *E coli, Staphylococcus aureus, Streptococcus pyogenes* and *erobacter aerogenes* are usually susceptible, while Proteus species, *Pseudomonas aeruginosa* and *Streptococcus faecalis* are usually resistant. Optimum effectiveness occurs when the urine pH is approximately 5. NFT is a synthetic nitrofuran derivative antibacterial agent. It is used for treatment of initial or recurrent urinary tract infections caused by susceptible pathogens. Adverse effects may include nausea, vomiting, loss of appetite, diarrhea, chest pain, chills, cough, fever, troubled breathing, sore throat, unusual weakness, dizziness, drowsiness, headache, and brownish discoloration of urine [28]. It is available in the following dosage forms; capsule, tablet and oral suspension [29].

1.5.3 Furazolidone

Furazolidone (FZD) was added to Annexe 4 of Regulation 2377/90 on 26 June 1995. This decision was taken because furazolidone residues, at whatever limit, in foodstuffs of animal origin constitute a hazard to the health of the consumer. It is known to be a genotoxic carcinogen and insufficient data are available concerning the identity and toxic potential of compounds released from bound furazolidone residues. Furazolidone therefore joins the other nitrofuran drugs; growth promoting hormones, such as diethylstilboestrol and trenbolone; and other antibacterial compounds, such as chloramphenicol, which are banned in the EU. It is often added to feeds to stimulate growth and prevent and control a number of diseases in animals. Depending on the intended use, the concentrations used vary from 8 to 400 mg/kg. For example, when fed to pigs at 100 mg/kg, furazolidone can help prevent bacterial enteritis due to *Salmonella* spp. Chickens and turkeys may be fed continuously at the rate of 8-10 mg/kg to stimulate growth and improve feed conversion [30].

1.6 Literature Review

Several analytical techniques have been reported to determine nitrofuran residues in various sample matrices have been developed such as in honey, muscle tissue, chicken meat and animal feeds as shown in Table 1.3.

It has not been yet reported for determination of FZD, NFT and NFZ by using microflow injection incorporating chemiluminescence detection (μFI-CL). Accordingly, the purpose of this research is to design and construct the μFI-CL system with the micellar media as surfactants on the chemiluminescence reaction of lumimol-hydrogen peroxide system.

Table 1.3 A brief of the methods for the determination of nitrofurans

Sample	Analyte	Techniques	Linear range ($mg\ L^{-1}$)	r^2	% Recovery	LOD ($mg\ L^{-1}$)	LOQ ($mg\ L^{-1}$)	Reference
Pharmaceutical formulations	NFZ	Spectrophotometric method	8.0-50.0	-	99.98	-	-	[31]
Pharmaceutical preparations	NFZ NFT FZD	HPLC	1×10^{-3}- 10×10^{-3} 1×10^{-3}- 10×10^{-3} 3×10^{-3}- 18×10^{-3}	0.9992 0.9994 0.9992	99.97 ± 1.44 99.94 ± 1.34 99.93 ± 1.61	-	-	[32]
Pharmaceutical formulations	FZD	Continuous and Stopped flow FIA-spectrophotometry	1-30	0.9994 0.9975	-	0.20 0.27	0.67 0.90	[33]
Pharmaceutical preparations	NFZ FZD	Colorimetric method	1.0×10^{-3}-8.0×10^{-3} 1.0×10^{-3}-8.0×10^{-3}	0.9999 0.9999	98.9 ± 0.3 to 100.4 ± 0.7	-	-	[34]
Drug	NFZ NFT FZD	Thin layer and high performance liquid chromatography (TLC-HPLC)	0.1-5.0	0.989 0.996 0.977	-	0.015 0.022 0.060	-	[35]
Suspension drug	FZD	High performance thin layer chromatographic (HPTLC) and high performance liquid chromatographic (HPLC)	3.5-17.5 10.5-63	0.998 0.999	99.51 99.91	-	-	[36]
Pharmaceutical preparations	FZD	Bivariate spectrophotometry	2.0-12.4	0.9996	100.4 ± 0.8	0.12	-	[37]

Table 1.3 Continued

Sample	Analyte	Techniques	Linear range ($mg\ L^{-1}$)	r^2	% Recovery	LOD ($mg\ L^{-1}$)	LOQ ($mg\ L^{-1}$)	Reference
Antibacterial drug	NFT	Cathodic stripping voltammetry	1.0×10^{-9}-9.0×10^{-8} M	0.9904	99.80	1.0×10^{-9} M	-	[38]
Pharmaceutical formulations	NFT	Square-wave cathodicadsorptive stripping voltammetry	1×10^{-8} -2×10^{-7} M	0.996	101.49±0.65	1.32×10^{-10} M	4.4×10^{-10} M	[39]
Pharmaceutical preparations Blood plasma Urine	NFZ	FI-CL (NBS reagent with H_2O_2)	1.0×10^{-7}-1.0×10^{-5}	0.9998	- 98.2 ± 4.3 100 ± 5.5	2×10^{-8}	-	[40]
Pharmaceutical preparations	NFZ NFT	Spectrofluorimetric method	0.004-0.050 0.02-0.24	0.99986 0.99991	-	0.3×10^{-3} 1.9×10^{-3}	1.0×10^{-3} 6.3×10^{-3}	[41]
Animal feeds	FZD	LC-UV	5-125 mg/kg	0.9999	93.8	1 mg kg^{-1}	-	[42]
Animal feeds Farm water	NFZ NFT FZD	HPLC-DAD	0.8×10^{-3}-100×10^{-3} 1.0×10^{-3}-130×10^{-3} 0.8×10^{-3}-100×10^{-3} -	0.9999 0.9997 0.9998 -	88 ± 3.7 85 ± 4.4 78 ± 4.7 95 ± 2.9 91 ± 3.0 81 ± 3.6	2.1 μg kg^{-1} 2.6 μg kg^{-1} 2.2 μg kg^{-1} 0.21×10^{-3} 0.26×10^{-3} 0.22×10^{-3}	- 0.71×10^{-3} 0.85×10^{-3} 0.72×10^{-3}	[43]
Animal feeds	NFZ NFT FZD	HPLC-DAD	10×10^{-3}-100×10^{-3}	0.981 0.985 0.992	-	-	< 1.0	[44]

Table 1.3 Continued

Sample	Analyte	Techniques	Linear range (mg L^{-1})	r^2	% Recovery	LOD (mg L^{-1})	LOQ (mg L^{-1})	Reference
Animal feeds	FZD	Flow-injection Post-chemiluminescence (FI-CL)	1.0×10^{-7}-1.0×10^{-5}	0.9949	87.0	1.96×10^{-8}	-	[45]
Animal feeds	NFZ	Flow injection chemiluminescence (FI-CL)	0.5–8	0.9968	96.67 $\pm$ 0.83	0.25 mg kg^{-1}	0.83	[46]
	NFT			0.9952	95.26 $\pm$ 1.30	0.25 mg kg^{-1}	0.83	
	FZD			0.9887	94.95 $\pm$ 0.58	0.25 mg kg^{-1}	0.83	
Chicken eggs	NFZ	HPLC-DAD	0.10-2.50	0.9969 to	85.0-85.7	2.5	-	[47]
	FZD			0.9981	87.3-88.9	5.0		
Milk	NFT	HPLC-coulometric detection	10 $x10^{-3}$- $60x10^{-3}$and	0.9967	95.0 $\pm$ 2.0	4 $x\ 10^{-3}$	-	[48]
	FZD		0.1-2.2	0.9268	97.0 $\pm$ 6.0			
Honey	NFZ	LC-MS-MS	1 $x\ 10^{-3}$- 20 $x\ 10^{-3}$	>0.9996	-	1.1 $x\ 10^{-3}$	3.8 $x\ 10^{-3}$	[48]
	NFT		1 $x\ 10^{-3}$- 20 $x\ 10^{-3}$	>0.993		2.1 $x\ 10^{-3}$	7.1 $x\ 10^{-3}$	
	FZD		1 $x\ 10^{-3}$- 10 $x\ 10^{-3}$	>0.995		1.0 $x\ 10^{-3}$	3.3 $x\ 10^{-3}$	
Turbot fish	NFZ	Microemulsion electrokinetic chromatography (MEEKC)	1.0-140	0.9958	96.7-104.5	0.5	-	[50]
	NFT		10-425	0.9945	91.6-100.3	2.0		
	FZD		15-300	0.9989	94.6-98.7	2.0		

1.7 Research Aims

In this thesis has aimed to develop a microfluidic system combined with chemiluminescence procedure for the determination of some nitrofurans such as nitrofurazone (NFZ), nitrofurantoin (NFT) and furazolidone (FZD). This proposed method is aimed to gain a small amount of chemical consumption, simple and rapid instrumentation for the determination of some nitrofurans in pharmaceutical preparations and animal feeds. The aims of this research can be summarized as follows:

To design and fabricate a microfluidic device with chemiluminescence system for the determination of NFZ, NFT and FZD.

To apply a microflow injection chemiluminescence procedures for quantitative analysis ofNFZ, NFT and FZD in pharmaceuticals and animal feed samples.