CHAPTER IV

CONCLUSION AND SUGGESTION FOR FURTHER WORK

4.1 Conclusion

A microfluidic device combined with chemiluminescence (CL) detection in the continuous-flow system was developed for the determination of some nitrofurans such as nitrofurazone (NFZ), nitrofurantoin (NFT) and furazolidone (FZD). The microfluidic chemiluminescence systems were fabricated from easily available materials and instruments. The proposed method presents good reproducibility (1.50 - 2.60 % RSD), high sensitivity (0.058 - 0.12 mg L^{-1}), and sample throughput (34 - 40 h^{-1}). The method is also simple, inexpensive and reliable. This method was successfully applied to the determination of nitrofurans in pharmaceutical preparations and animal feeds.

This research work consists of two parts. The first was tested for the sensitivity of chemiluminescence reactivity of some nitrofurans (NFZ, NFT and FZD) by a simple flow injection chemiluminescence method (FI-CL), which is based on CL light induced by luminol in an alkaline medium. The flow injection configuration used for the determination of nitrofurans was designed to provide reaction conditions for magnifying and enhancing effect on the CL generated by the reaction of luminol/potassium ferocyanide ($K_4Fe(CN)_6$) in the presence of Triton-X100/hydrogen peroxide (H_2O_2) media as described in Figure 2.4. This method involves the injection of each nitrofurans (NFZ, NFT and FZD) standards into pure water carrier stream,

which then is merged at a T-piece with stream of Triton-X100/H_2O_2 solution. The reagent stream is consisting of luminol/($K_4Fe(CN)_6$ in alkaline medium. In aqueous solutions, the most commonly used chemiluminescent species is luminol (5-amino-2,3-dihydrophthalazine-1,4-dione), which reacts with H_2O_2 in the presence of a catalyst ($K_4Fe(CN)_6$) in alkaline solution to yield 3-aminophthalate in an excited electronic state which returns to ground state with the production of light [62-63, 66-67]. The light intensity can easily be monitored with a photomultiplier tube with no wavelength discrimination, then the intensity of enhanced emission is proportional to the concentration of nitrofurans; thus the amount of nitrofurans can be determined by measuring the increase in CL intensity. This CL reaction seems promising as a basic for development of a novel microflow analysis procedure for nitrofurans determination.

The second, the good sensitivity obtained by the proposed flow injection chemiluminescence system led to further investigation to develop the microfluidic injection device. The microfluidic device was adopted for development of a microfluidic chemiluminescence procedure for some nitrofurans determination. The microfluidic device was designed using *CorelDraw X4*, and was fabricated on an acrylic glass by laser engraving. The dimension of the flow channels was shown in Figure 3.2. The effect of the dimension of flow channels (the width of the channels was just changed) on the CL intensity was investigated. The dimension of the flow channels were 4.00 cm in length (transport of all) and 400 μm in width, which led to small volume of solvent consumptions with good sensitivity. This method was more sensitive than those obtained in the previous works on flow injection (FI) system by Díaz et al. [33], Thongsrisomboon et al. [46] and Du et al. [40]. A series of experiments were conducted to establish the optimum analytical variables. The parameters optimized include reagent

concentrations and some manifold parameters. The optimum conditions were obtained by physical and chemical parametric illustrated in Table 3.15. Under the optimum conditions, linear calibration graphs over the same range of 0.30 - 9.00 mg L^{-1} for these nitrofurans were established. The limits of detection detection, defined as three times the standard deviation of the noise (S/N=3) was determined for NFZ, NFT and FZD which were found to be 0.058, 0.11 and 0.12 mg L^{-1} respectively, which were better than those previous by reported by Díaz et al. [33] and Thongsrisomboon et al. [46]. The quantitation limit (S/N=10) for NFZ, NFT and FZD were found to be 0.19, 0.36 and 0.40 mg L^{-1}, respectively. The precision of the system was evaluated by injecting 100 μL volume of each standard nitrofurans (2.0 mg L^{-1}) into the microflow system. The percentage relative standard deviation (% R.S.D) were found to be 1.80%, 1.50% and 2.60% for NFZ, NFT and FZD, respectively (n = 15). The intra-day variations were found to be 1.36%, 3.21% and 2.22% for NFZ, NFT and FZD, respectively. The inter-day variations were found to be 2.87%, 2.77% and 2.91% for NFZ, NFT and FZD, respectively. Comparison of the proposed μFI-CL method with the selected earlier reported methods for determining nitrofurans indicated that the proposed method was more sensitive than the flow injection analysis (FIA) by Thongsrisomboon et al. [46] and photometric flow injection analysis by Díaz et al. [33]. Furthermore, the proposed μFI-CL method possesses simple set-up, minimum reagent consumption, minimum wastes generation, and increases the potential for miniaturization and automation. It plays an important role for development into a green analytical method for the modern analytical chemist society as depicted in Table 4.1.

The proposed method had been applied to determine pharmaceutical preparations and animal feeds in real samples by using solid phase extraction (SPE) for sample

clean-up, and the results were compared favorably with those obtained by using the official BP or HPLC method (Table 3.26). In contrast to pharmaceutical and animal feed matrices, SPE cartridges were used as a pretreatment for cleaning FZD blend tablet, FZD suspension and animal feed samples and concentrating the nitrofurans. The concentrated nitrofurans was removed from the column with an eluting solution, and then analyzed for nitrofurans by the proposed and reference methods [53-54, 56]. A Student two-tail paired *t*-test was also applied yielding a value, which was less than the theoretical value of 2.776 for a confidence interval of 95%. It may thus be concluded that there are no significant differences between the proposed method and the reference procedure. The recoveries of proposed method were between 95.48 - 105.80%. This proposed method shows a short analysis time, a small amount of chemical consumption with minimum waste production and a good reproducibility, which provides a simple, rapid, inexpensive instrumentation and acceptable method for the determination of nitrofurans.

4.2 Suggestions for Further Work

On-line extraction or preconcentration (an interesting way to improve the performance of the determination and to enhance the sensitivity and low detection limit) and separation of the analytes are alternative techniques which make it suitable for sample extraction from many substances normally present in pharmaceutical preparations and animal feeds, such as kaolin and neomycin which may cause interfering effects.

The possibility of using a new by designed microfluidic chemiluminescence flow cell, because its combination affects the time-dependent variations in the intensity of light emission. Two important factors in any chemiluminescence determination are the mixing of the reagents and the detection cell. The reagents must mix satisfactorily in order to obtain the maximum light emission.

This proposed method may be possible for determining nitrofuran metabolite residues in a wide range of various sample matrices such as biological, agricultural, samples including foods, feeds and water samples.

Table 4.1 Comparison of the analytical figures of merit of the proposed μFI-CL method with some earlier reported methods

Parameters	**Reference**			**Propose method**
	[40]	**[46]**	**[33]**	
Techniques	FI-CL	FI-CL	FI-spectrophotometry	μFI-CL
Analyte	NFZ	NFZ	FZD	NFZ
		NFT		NFT
		FZD		FZD
Sample	Pharmaceutical preparations Biological fluids	Animal feeds	Pharmaceutical formulations	Pharmaceutical preparations Animal feeds
Reagent	NBS	$KMnO_4$	NaOH solution with 1% (v/v) of DMF	Luminol
Regression equation[a]	*I = 6.2C + 0.78*	*I = 8.0407C - 2.7899*	-	*I = 53.764C - 3.894*
		I = 9.3475C + 0.5028		*I = 28.551C - 4.5994*
		I = 10.269C + 5.6963		*I = 20.222C + 3.4745*
Correlation coefficient (r^2)	0.9998	0.9968	0.9994	0.9997
		0.9887		0.9992
		0.9952		0.9990
Detection limits[b]	2×10^{-8}	0.25	0.20	0.058
(mg L^{-1})		0.25		0.11
		0.25		0.12
Quantitation limit	-	0.83	0.67	0.19
(mg L^{-1})		0.83		0.36
		0.83		0.40

Table 4.1 Continued

Parameters	**Reference**			**Propose method**
	[40]	**[46]**	**[33]**	
Linear range (mg L^{-1})	1.0×10^{-7} – 1.0×10^{-5}	0.5–8.0	1-30	0.30 – 9.00
Reagents consumption (mL min^{-1})	2.10	3.50	1.00	0.15
Inter-day precisions	-	1.7	-	2.87
(%RSD)		2.94		2.77
		1.37		2.91
Intra-day precisions	-	1.88	-	1.36
(%RSD)		2.91		3.21
		1.94		2.22

[a] *I* is the analytical signal in mV and C is the concentration of each nitrofurans (mg L^{-1}).
[b] The limit of detection, calculated at three times the standard deviation of the noise.