CHAPTER III

RESULTS AND DISCUSSION

In this chapter, a microfluidic system combined with chemiluminescence detection procedure has been described for the determination of some nitrofurans such as nitrofurazone (NFZ), nitrofurantoin (NFT) and furazolidone (FZD). Optimum conditions for the determination of NFZ, NFT and FZD were thoroughly investigated. The proposed method was applied successfully for the determination of NFZ, NFT and FZD in pharmaceutical preparations and animal feeds. Evaluation of proposed method was also carried out by comparing the results obtained with those obtained by the reference method (the official BP and HPLC method) [58,56].

3.1 Preliminary Studies by Using Simple Flow Injection Chemiluminescence Method for Determination of Some Nitrofurans

In the first session, for testing the sensitivity of chemiluminescence reactivity was investigated for some nitrofurans (furazolidone, nitrofurantoin and nitrofurazone) by a simple flow injection chemiluminescence method. The method is based on CL induced by luminol in alkaline medium solution, it have been used to generate CL intensities from a wide range of inorganic and organic compounds [60].

In order to obtain some ideas on the possible mechanism of the CL reaction, the relevance of the luminol as analytical CL reagent does not rely on the emission efficiency but on possibilities that many difference species can influence the

mechanism and the kinetics of the indicator reaction (Figure 3.1). The analyte can act as catalyst whose concentration may influence the intensity or amount of emitted light. It was reported that singlet oxygen ($^1O_2$) could be generated during the reaction between luminol and H_2O_2. Therefore, it was concluded that singlet oxygen was involved in the CL reaction [40,64].

The luminol-based CL reaction is a well-known method for the detection of reactive oxygen species, such as $O_2^{\bullet-}$, $^1O_2$ and H_2O_2, because these species react quickly with luminol in alkaline solution to emit light, as the hydroperoxide intermediate of luminol decomposes into aminophthalate. According to the well-known mechanism the supposed emitter is excited 3-aminophthalate anion whose maximum emission occurs at 425 nm.

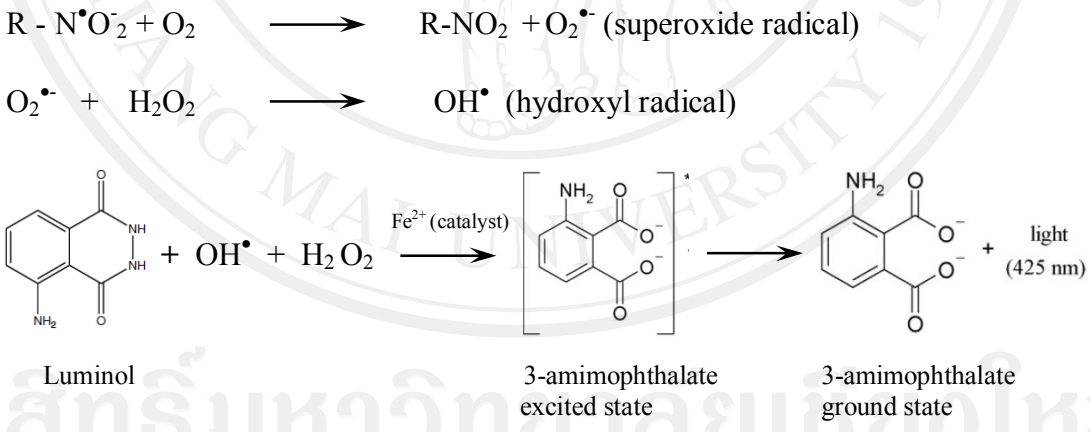

Figure 3.1 Scheme of the suggested CL reaction mechanism

Preliminary experiments implemented using a simple flow injection chemiluminescence method to determine nitrofurans, this method involves the injection of nitrofuran samples or standards into ultrapure water carrier stream, which then merges at a T-piece with a stream of TritonX-100/hydrogen peroxide (H_2O_2) solution. The reagent stream consisting of luminol/potassium ferocyanide ($K_4Fe(CN)_6$) in alkaline medium. The elicited chemiluminescence intensity of the resulting reaction mixture was measured by photomultiplier tube operated at a voltage of 800 V. The designed flow injection manifold was a three channel manifold (Figure 2.4) in which the pure water (C), TritonX-100/H_2O_2 (R1) and luminol/$K_4Fe(CN)_6$ in alkaline medium (R2) were premixed in the reagent reservoir prior to propelling into the flow system. The concentration of NFZ, NFT and FZD was quantified by the CL intensity (peak height).

The preliminary experimental results as shown in Table 3.2 and Figure 3.2. It was found that the CL signal generated by the reaction between luminol/$K_4Fe(CN)_6$ in alkaline medium. The working ranges and the corresponding optimal values are presented in Table 3.1. Maximum CL intensity was obtained when the standard solution (NFZ, NFT and FZD) was injected to pure water carrier stream and then mixed with H_2O_2 in surfactant media solution, the reaction mixture was then merged with a reagent stream consisting of luminol and $K_4Fe(CN)_6$ in NaOH solution before reaching the detector. Proposed FI-CL system will be used as a basic for development of a microfluidic system working at under the optimum conditions for quantitative analysis of nitrofurans in pharmaceutical preparations and animal feeds samples.

Table 3.1 Optimized operating conditions for the determination of nitrofurans by a simple flow injection chemiluminescence system

Parameter	Range studied*	Optimal value
PMT voltage (V)	600 – 1000	850
Luminol concentration (mmol L^{-1})	0.50 – 1.00	0.85
$K_4Fe(CN)_6$ concentration (μmol L^{-1})	10 – 100	40
NaOH concentration (mol L^{-1})	0.20 – 0.65	0.45
H_2O_2 concentration (mol L^{-1})	0.001 – 2.00	0.10
Flow rate C (mL min^{-1})	0.1 – 1.7	1.3
Flow rate R1 (mL min^{-1})	0.1 – 1.7	0.3
Flow rate R2 (mL min^{-1})	0.1 – 1.7	0.3
Triton X-100 concentration (% v/v)	0.01 – 0.50	0.3

* NFZ was used for parameter studied.

Table 3.2 Calibration data obtained from a simple flow injection chemiluminescence method for triplicate injections

Nitrofurans concentration (mg L^{-1})	CL intensity (mV) ± S.D.		
	NFZ	NFT	FZD
0.03	18.3 ± 0.6	ND**	ND**
0.05	25.0 ± 0.0	ND**	ND**
0.07	28.0 ± 1.0	ND**	ND**
0.09	26.3 ± 0.6	ND**	ND**
0.10	32.7 ± 0.6	27.3 ± 0.6	16.7 ± 2.5

Table 3.2 Continuous

Nitrofurans concentration (mg L^{-1})	CL intensity (mV) ± S.D.		
	NFZ	**NFT**	**FZD**
0.50	120.7 ± 1.5	84.7 ± 2.5	49.7 ± 1.5
1.00	259.7 ± 3.8	162.3 ± 0.6	107.7 ± 1.2
1.50	408.0 ± 4.4	254.0 ± 4.0	159.7 ± 2.3
2.00	582.3 ± 1.2	373.0 ± 3.5	225.7 ± 1.2
2.50	-	467.3 ± 8.4	289.7 ± 3.5
3.00	-	580.3 ± 6.7	365.7 ± 4.2
3.50	-	-	435.0 ± 4.6

**Not detected

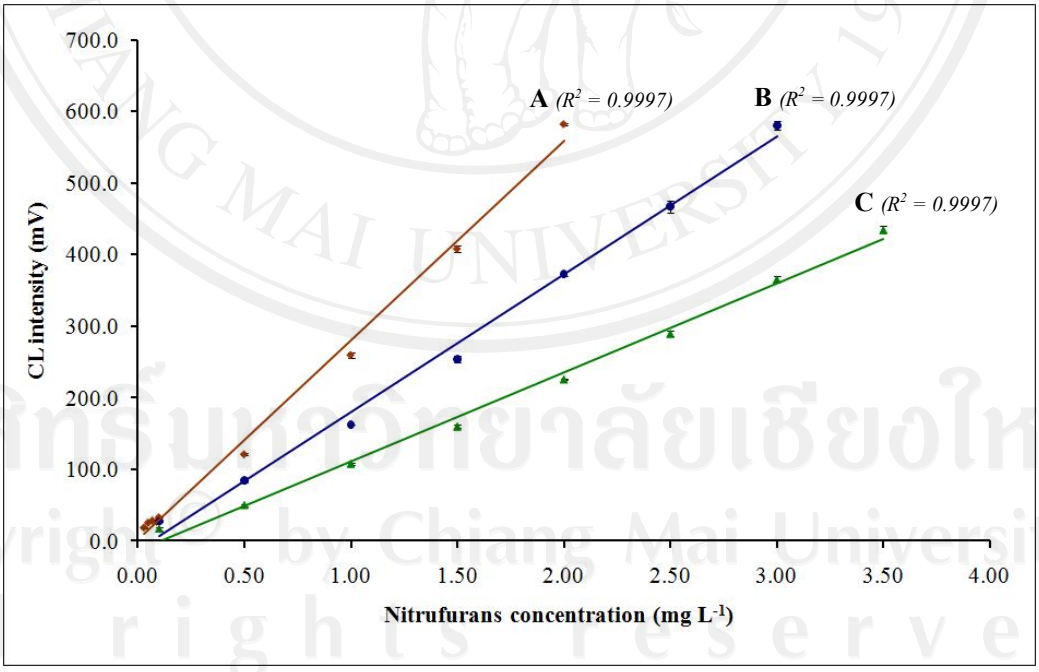

Figure 3.2 Calibration plots obtained from a simple flow injection chemiluminescence method for triplicate injections of each NFZ (A), NFT (B) and FZD (C) standards. Conditions: as describe in table 3.1

3.2 Microchip Designs

The choice of a starting substrate material for a microfluidic device dictates the processes that can be used for microfabrication. Factors to consider when choosing a substrate include temperature limits (many standard processes involve heat), chemical resistance (etching relies on caustic solutions and gases), and mechanical strength (bonding and substrate handling are often required). This research, the microchip was fabricated by using poly(methylmethacrylate) (PMMA) substrates and the base plate was etched by means of laser engraving. PMMA is a good candidate for the system. It is heat resistant, chemically stable, and transparent, which makes it a good light transmitter for monitoring.

In this research, study was to find an optimal microfluidic channels with a built in flow cell of the microfluidic system to achieve the best sensitivity in chemiluminescence analysis. The dimension of the channel has great effect on the CL intensity.

Firstly, the effect of the dimension of flow cell on CL intensity was investigated. The dimension of the flow channels was shown in Figure 3.3. The width of the channels on the microfluidic device was waried (Figure 3.3A insert a) with three sizes i.e., 0.30, 0.40 and 0.50 mm. It was indicated that the relative CL intensity increased with the increasing width of the flow channel. If the channel width was smaller than 0.30 mm, the resistance (back pressure) in the microchannels increased obviously. So the channel width of 0.40 mm was used.

The microchip layout has two inlets and one outlet channel: one inlet is used for the injection of sample into deionized water carrier stream, which is then merged with a oxidant/surfactant stream of hydrogen peroxide and sodium hexametaphosphate

(SHMP) solution (1.50 cm in length and 400 μm in width). Other inlet is used for reagent stream consisting of luminol and potassium ferrocyanide (K_4FeCN_6) in an alkaline medium (1.50 cm in length and 400 μm in width), and finally an outlet channel for the waste. The channel layout consists of channels with equivalent width and depth, but different length as shown in Figure 3.3A (insert a). The two inlets channels are merged at reaction area with the time specified of the chemical reactions. The flow rate was around 0.15 – 0.30 mL min^{-1}, with low reagent and sample consumption.

Finally, the reaction mixture is transport in the Zig Zag pattern flow channel, where the CL light intensity occurs and is monitored by means of a photomultiplier tube (PMT) connected to a personal computer.

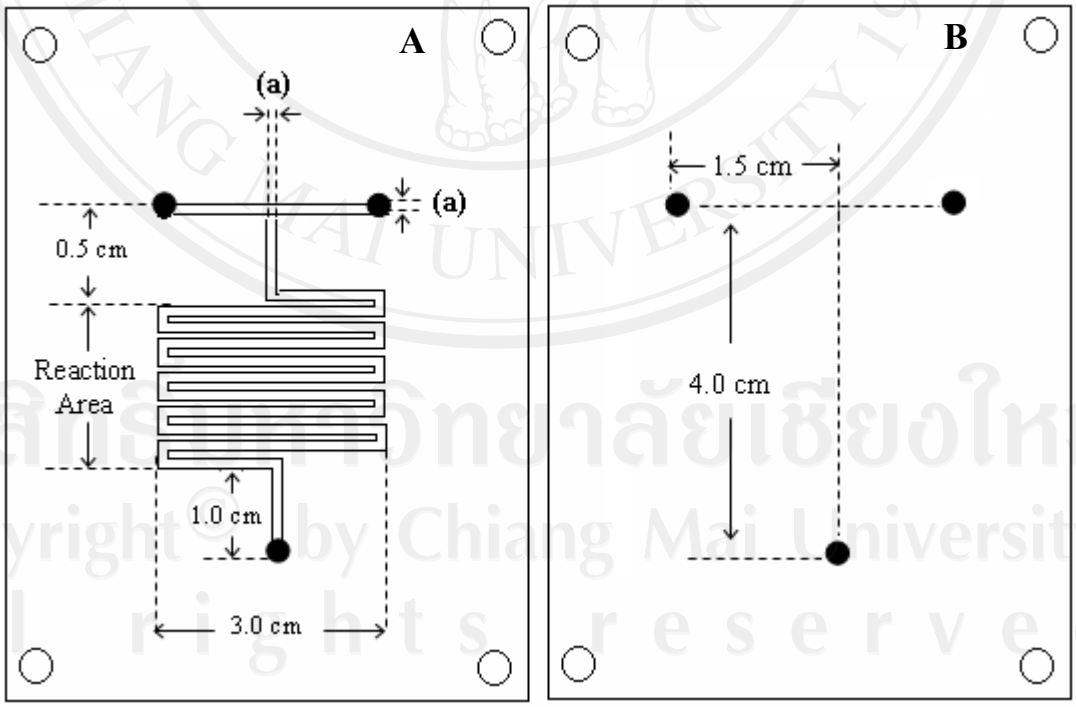

Figure 3.3 The dimensions of the microchannels on the microfluidic device. Base plate (A); Top plate (B)

3.3 Microfluidic Chemiluminescence for Determination of Some Nitrofurans in Pharmaceutical Preparations and Animal Feeds Samples

According to the preliminary investigation described in session 3.1, a flow injection chemiluminescence method to determine nitrofurans was likely to be miniaturized for a small scale lab. It was possible for developing microfluidic system for nitrofurans analyses due to its better reducing the reagent consumption with minimum waste production. The designed microfluidic device was combined with chemiluminescence detection in the continuous-flow system for the determination of NFZ, NFT and FZD. The analytical characteristics such as linear calibration ranges, the detection limits (LOD), and the limits of quantification (LOQ) for NFZ, NFT and FZD determination were investigated after the optimum conditions were obtained with the lab-on-chip analysis.

3.3.1 Optimization of the microfluidic chemiluminescence system by univariate method

The flowing parameters for the determination of nitrofurans by the microfluidic chemiluminescence method were optimized including the chemical and physical variables, in an effort to obtain maximum CL intensity. The optimum conditions were investigated by means of the univariate optimization procedure (changing one variable in turn and keeping the others at their optimum values). All optimum values were chosen by judging by compromising the peak height, stability of the base line, low or no positive blank signals, low analysis time, availability and economy. To optimize the conditions, the microfluidic chemiluminescence system manifold in Figure 2.5 and the preliminary experimental conditions (Table 2.1) were used.

3.3.1.1 Effect of photomultiplier tube (PMT) voltage on the CL intensity

The influence of photomultiplier tube (PMT) voltage was studied firstly to search for an optimal input voltage. The voltages ranging from 750 to 1,000 V were tested, noting that the maximum input voltage recommended by manufacturer was 1,000 V. The experiments were performed with multiple injection of standard solution of each nitrofurans (NFZ, NFT and FZD) at concentration of 2.00 mg L^{-1}, made up in a carrier stream. The three channels peristaltic pump was used to propel a carrier stream, reagent streams and a stream of oxidant/surfactant solution. The total flow rate was set at 0.8 mL min^{-1}. The results were shown in Table 3.3 and Figure 3.4. The potential of the power supply was increased stepwise and the current representing CL intensity (in mV) was measured after an injection of NFZ, NFT and FZD at each potential step. As aspect, both noise and analytical signal increased as the PMT voltage increased. The resulting plot of CL signal-to-noise ratio reached a maximum value at 900 V, which was selected for subsequent experiments.

Table 3.3 Effect of photomultiplier tube applied voltage on the CL intensity of NFZ, NFT and FZD

$E_{applied}$	CL intensity (mV)* ± S.D.		
(V)	NFZ	NFT	FZD
750	19.0 ± 0.0	6.0 ± 0.0	5.0 ± 0.0
775	23.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0
800	29.0 ± 0.0	10.0 ± 0.0	8.0 ± 0.0
825	38.0 ± 0.0	13.0 ± 0.0	10.0 ± 0.0
850	49.0 ± 0.0	13.3 ± 0.0	10.8 ± 0.0
875	50.3 ± 0.6	16.7 ± 0.0	11.3 ± 0.0
900	62.5 ± 1.0	23.5 ± 0.6	18.8 ± 0.6
925	48.3 ± 0.6	16.2 ± 0.6	14.7 ± 1.2
950	50.3 ± 0.6	15.3 ± 0.6	15.8 ± 0.6
975	45.4 ± 1.5	14.4 ± 0.6	14.4 ± 0.6
1000	51.0 ± 1.5	16.6 ± 1.5	14.9 ± 0.6

*average of triplicate results

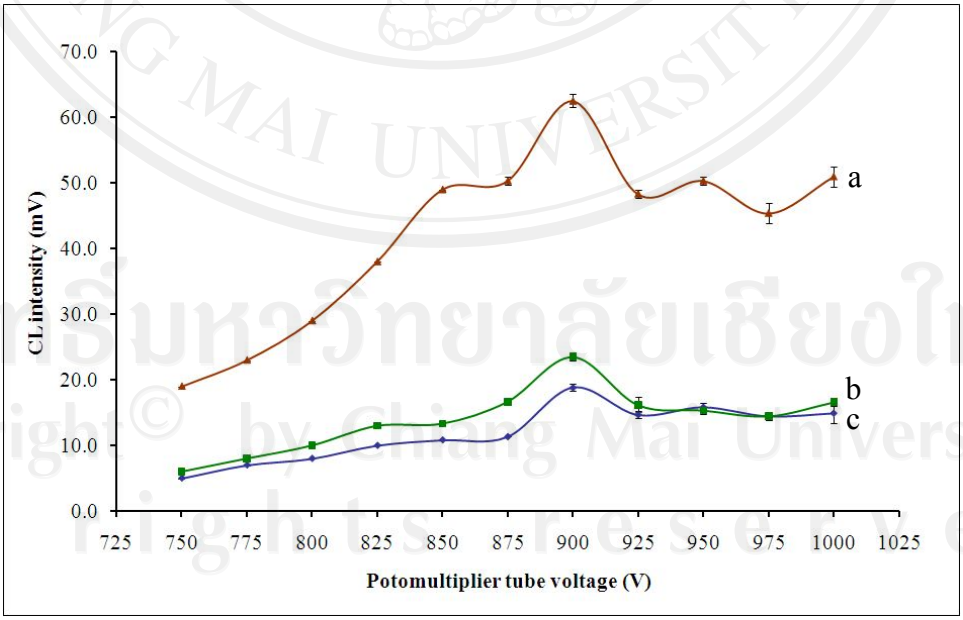

Figure 3.4 Effect of photomultiplier tube voltage on the CL intensity of NFZ (a), NFT (b) and FZD (c)

3.3.1.2 Effect of different surfactants on the CL intensity

Due to various unique and advantageous properties of surfactants, which should better facilitate analytical CL measurement, there are a number of reports [61-62] on the application of various kinds of surfactant in CL measurement such as Triton X-100, Tween 80, sodium hexametaphosphate (SHMP), sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB). Figure 3.5 and Table 3.4 show the effect of each surfactant (TritonX-100, Tween 80, SHMP, SDS and CTAB) on CL intensity. Each surfactant was added with H_2O_2 solution and used as oxidant/ surfactant stream solution. The surfactant media of SHMP shows dramatically increasing in CL intensity over other oxidant/surfactant streams (TritonX-100, SDS, Tween 80 and CTAB). The effects of surfactant media on emission intensity in luminol CL system were investigated. It was found that useful improvements in both signal intensity and signal-to-noise ratio applied to determine nitrofurans in pharmaceutical preparations were observed. Hence, SHMP was the most effective enhancing agent for the CL emission in this study and was used for further investigations. Figure 3.6 and Table 3.5 illustrates the CL intensity for NFZ, NFT and FZD signal at various concentrations of SHMP media. It is shown that, the 0.1 % (v/v) of SHMP media obviously exhibits the highest CL intensity, and was selected to mix with H_2O_2 in the oxidant/surfactant stream for further the microfluidic chemiluminescence system.

Table 3.4 Effect of surfactant variables on the CL intensity of NFZ, NFT and FZD

Types of surfactant	Surfactant concentration (% w/v)	CL intensity (mV)* ± S.D.		
		NFZ	NFT	FZD
Tween 80	0.00	64.7 ± 0.6	24.6 ± 0.6	17.8 ± 0.6
	0.01	55.0 ± 1.2	16.9 ± 0.6	13.9 ± 1.0
	0.05	40.5 ± 1.0	7.2 ± 1.2	13.7 ± 1.0
	0.10	23.0 ± 0.6	4.4 ± 1.2	9.5 ± 0.6
	0.20	14.8 ± 0.6	3.0 ± 0.6	6.0 ± 0.6
	0.30	9.1 ± 2.5	2.4 ± 1.0	3.9 ± 0.6
	0.50	4.0 ± 2.1	2.1 ± 1.2	2.6 ± 1.2
SHMP	0.00	77.7 ± 0.6	21.8 ± 0.6	22.1 ± 0.6
	0.01	80.7 ± 1.2	20.9 ± 0.6	25.3 ± 0.0
	0.05	95.3 ± 0.6	30.5 ± 0.6	29.3 ± 0.6
	0.10	120.0 ± 1.0	34.8 ± 0.6	29.2 ± 0.6
	0.20	71.1 ± 1.0	17.1 ± 1.5	14.2 ± 1.0
	0.30	66.0 ± 1.0	12.1 ± 1.0	11.6 ± 1.0
	0.50	49.9 ± 1.0	8.6 ± 1.5	9.2 ± 0.6
Triton x-100	0.00	76.3 ± 0.6	18.8 ± 0.6	23.8 ± 0.6
	0.01	84.5 ± 0.6	19.8 ± 0.6	22.6 ± 0.0
	0.05	86.8 ± 0.6	20.2 ± 0.6	21.6 ± 0.6
	0.10	73.5 ± 1.0	19.3 ± 0.6	17.5 ± 1.0
	0.20	74.2 ± 0.6	15.3 ± 0.6	14.3 ± 1.0
	0.30	79.8 ± 0.6	11.4 ± 0.6	13.2 ± 1.0
	0.50	63.7 ± 0.6	9.6 ± 0.6	10.1 ± 0.6

Table 3.4 Continued

Types of surfactant	Surfactant concentration (% w/v)	CL intensity (mV)* ± S.D.		
		NFZ	NFT	FZD
SDS	0.00	36.2± 1.5	27.0± 1.0	12.2± 0.6
	0.01	60.5± 1.5	28.8± 1.2	13.0± 0.0
	0.05	51.4± 1.0	29.0± 0.0	13.5± 1.0
	0.10	57.0± 1.7	27.8± 0.6	12.3± 0.6
	0.20	55.7± 1.2	26.3± 0.6	11.5± 0.0
	0.30	57.8± 0.6	26.3± 0.6	11.8± 0.6
	0.50	55.3± 1.0	25.3± 1.0	12.8± 0.6
CTAB	0.00	40.8± 0.0	30.5± 1.0	10.5± 0.0
	0.01	95.7± 0.6	23.0± 1.0	18.0± 0.0
	0.05	2.1± 0.0	N.D.**	N.D.**
	0.10	N.D.**	N.D.**	N.D.**
	0.20	N.D.**	N.D.**	N.D.**
	0.30	N.D.**	N.D.**	N.D.**
	0.50	N.D.**	N.D.**	N.D.**

*average of triplicate results

**Not detected

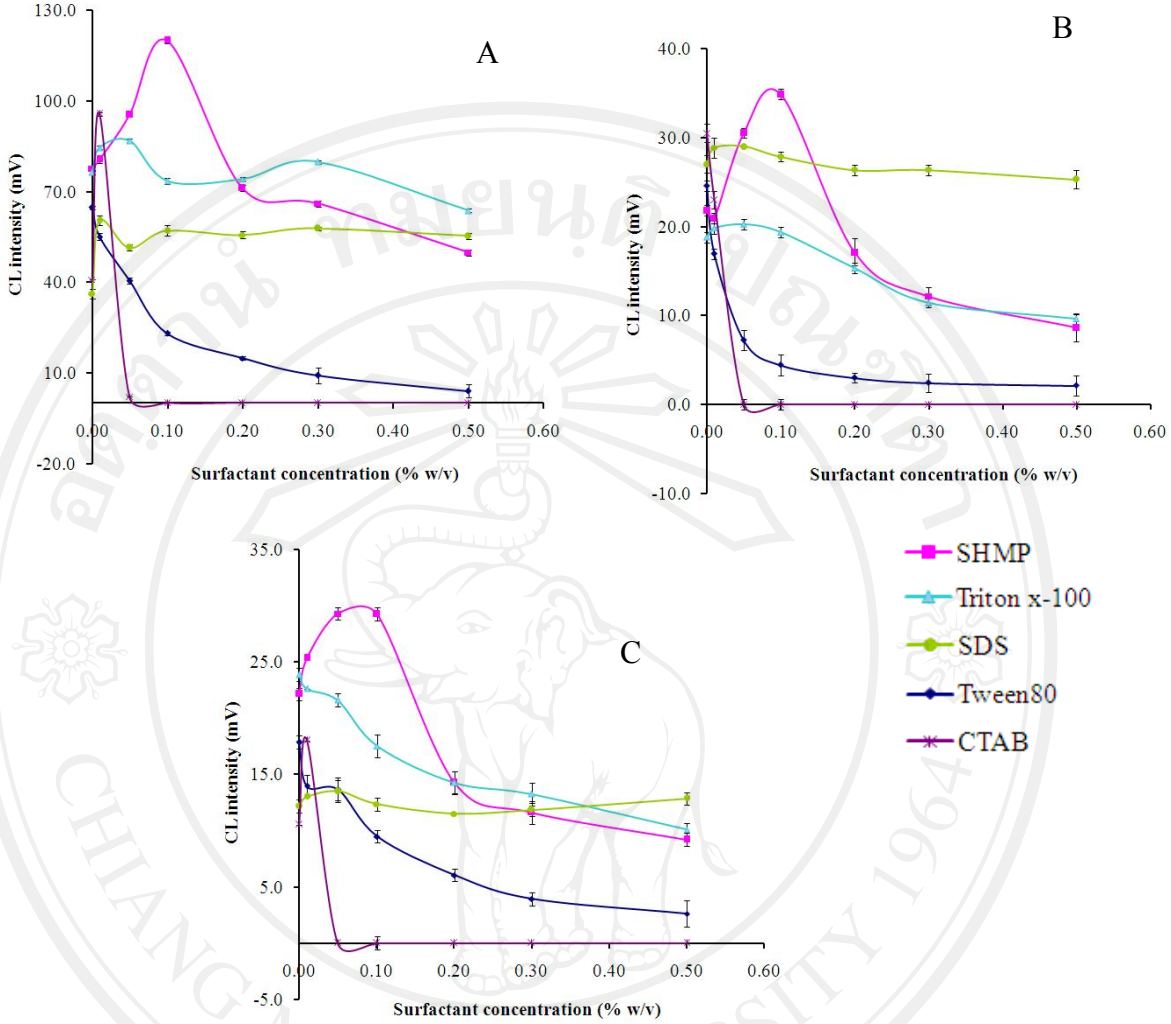

Figure 3.5 Effect of surfactant variables on CL intensity of NFZ (A), NFT (B) and FZD (C), each surfactant were mixed with H_2O_2 solution and used as oxidant/ surfactant stream solution.

Table 3.5 Effect of sodium hexametaphosphate concentration on the CL intensity of NFZ, NFT and FZD

SHMP concentration	CL intensity (mV)* ± S.D.		
(% w/v)	NFZ	NFT	FZD
0.00	60.0 ± 2.5	21.0 ± 0.6	16.0 ± 0.6
0.05	73.8 ± 1.5	36.0 ± 1.0	29.2 ± 1.5
0.10	162.3 ± 1.5	79.3 ± 0.6	45.0 ± 2.1
0.20	58.8 ± 0.6	44.5 ± 1.5	24.1 ± 0.6
0.30	28.3 ± 1.5	18.4 ± 0.0	17.0 ± 0.6
0.40	25.0 ± 1.2	14.0 ± 0.6	13.4 ± 1.5
0.50	17.8 ± 0.6	12.1 ± 1.5	11.6 ± 1.2

*average of triplicate results

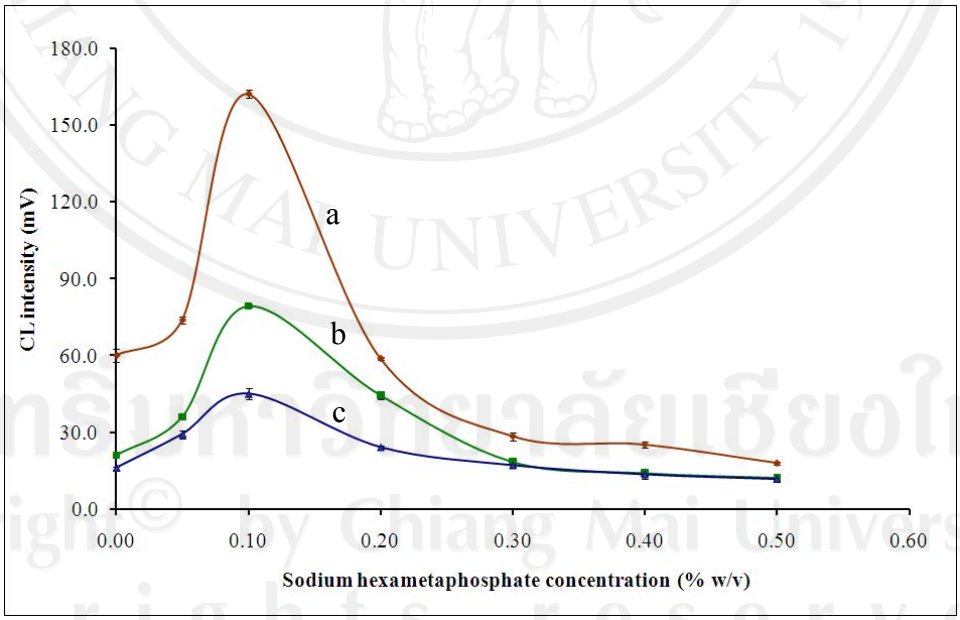

Figure 3.6 Effect of sodium hexametaphosphate concentration on CL the intensity of NFZ (a), NFT (b) and FZD (c)

3.3.1.3 Effect of different sensitizers on the CL intensity

In some cases the absence of a sensitizer, the luminol systems could only produce weak CL emissions. Thus, various compounds such as Quinnine, Fluorescin, and Rhodamine B were tested as potential sensitizers. The results are shown in Figure 3.7, clearly the CL intensity is significantly increased upon the addition of any sensitizers. Hence, it is not necessary to use these sensitizers for enhancing the CL intensity of nitrofurans. Table 3.6 shows the effect of different sensitizers on CL intensity of nitrofurans. It was found that all sensitizers showed little or no effect on CL intensity.

Table 3.6 Effect of sensitizers on the CL intensity of NFZ, NFT and FZD

Types of sensitizer	Nitrofurans concentration ($mg\ L^{-1}$)	CL intensity (mV)* ± S.D.		
		NFZ	NFT	FZD
None	1.00	75.0 ± 0.0	32.0 ± 0.0	19.0 ± 0.0
	1.50	127.0 ± 0.6	47.2 ± 1.0	28.7 ± 0.6
	2.00	177.7 ± 0.6	64.3 ± 0.6	41.0 ± 0.6
	2.50	223.3 ± 0.0	79.4 ± 1.0	56.0 ± 0.0
	3.00	284.7 ± 0.6	90.7 ± 0.6	67.3 ± 0.6
Quinnine	1.00	10.0 ± 1.0	10.6 ± 0.0	7.7 ± 1.0
	1.50	35.0 ± 0.6	25.5 ± 0.6	17.3 ± 0.6
	2.00	73.7 ± 0.0	40.0 ± 1.0	25.7 ± 0.0
	2.50	123.7 ± 0.0	55.7 ± 1.0	37.5 ± 0.0
	3.00	180.5 ± 0.6	65.8 ± 0.6	49.6 ± 0.6

Table 3.6 Continued

Types of sensitizer	Nitrofurans concentration ($mg\ L^{-1}$)	CL intensity (mV)* ± S.D.		
		NFZ	NFT	FZD
Fluorescin	1.00	15.0 ± 0.0	25.3 ± 1.0	10.6 ± 0.0
	1.50	67.0 ± 0.6	40.4 ± 0.6	19.5 ± 0.6
	2.00	110.0 ± 1.0	57.0 ± 1.0	28.0 ± 1.0
	2.50	163.0 ± 0.0	70.1 ± 0.0	40.7 ± 0.0
	3.00	220.3 ± 0.6	80.4 ± 0.6	60.5 ± 0.6
Rhodamine B	1.00	35.8 ± 0.6	29.4 ± 1.0	13.4 ± 0.6
	1.50	85.6 ± 1.0	43.5 ± 0.0	22.3 ± 1.0
	2.00	131.3 ± 0.6	60.0± 0.6	30.0± 0.6
	2.50	180.9 ± 0.0	73.6± 0.0	37.8± 0.0
	3.00	244.5 ± 0.6	85.2± 0.6	43.6± 0.6

*average of triplicate results

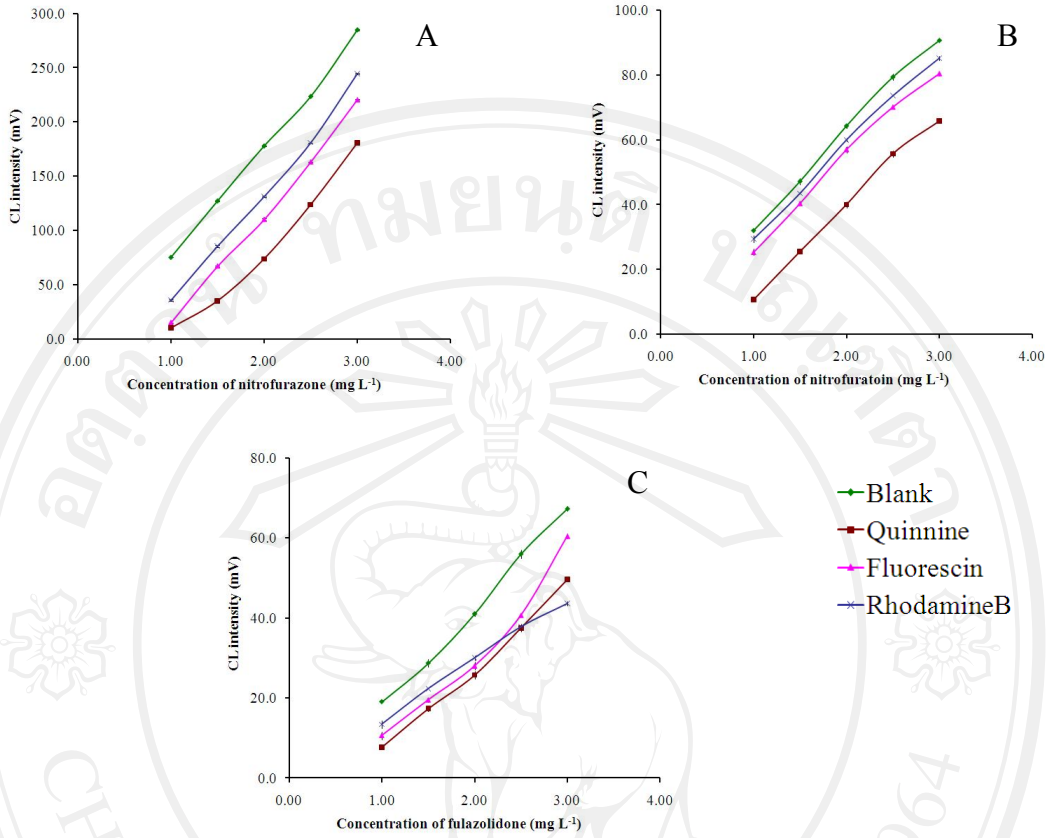

Figure 3.7 Effect of sensitizers on the CL intensity of NFZ (A), NFT (B) and FZD (C); Each sensitizer were added in a water carrier stream

3.3.1.4 Effect of luminol concentration on the CL intensity

The influence of varying concentrations of luminol between 0.5 and 2.0 mmol L^{-1} were examined (Figure 3.8 and Table 3.7). The highest intensity was obtained when the concentration of luminol was at 0.75 mmol L^{-1}, while $K_4Fe(CN)_6$ was set at 40 µmol L^{-1} and 0.45 mol L^{-1} NaOH (as the reagent stream), above which the intensity decreased gradually. Therefore, in order to achieve maximum sensitivity, 0.75 mmol L^{-1} luminol was used for the further studies.

Table 3.7 Effect of luminol concentration on the CL intensity of NFZ, NFT and FZD

Luminol concentration (mmol L^{-1})	CL intensity (mV)* ± S.D.		
	NFZ	NFT	FZD
0.50	30.8 ± 0.6	13.9 ± 0.0	9.3 ± 0.6
0.75	35.3 ± 0.0	18.4 ± 0.0	13.4 ± 0.6
1.00	26.4 ± 0.0	13.2 ± 0.6	7.3 ± 0.0
1.25	25.0 ± 0.6	12.8 ± 0.0	7.1 ± 0.6
1.50	23.9 ± 0.0	11.1 ± 0.6	6.2 ± 0.0
1.75	23.6 ± 0.6	10.5 ± 0.6	4.9 ± 0.0
2.00	21.1 ± 0.6	10.1 ± 0.6	4.3 ± 0.6

*average of triplicate results

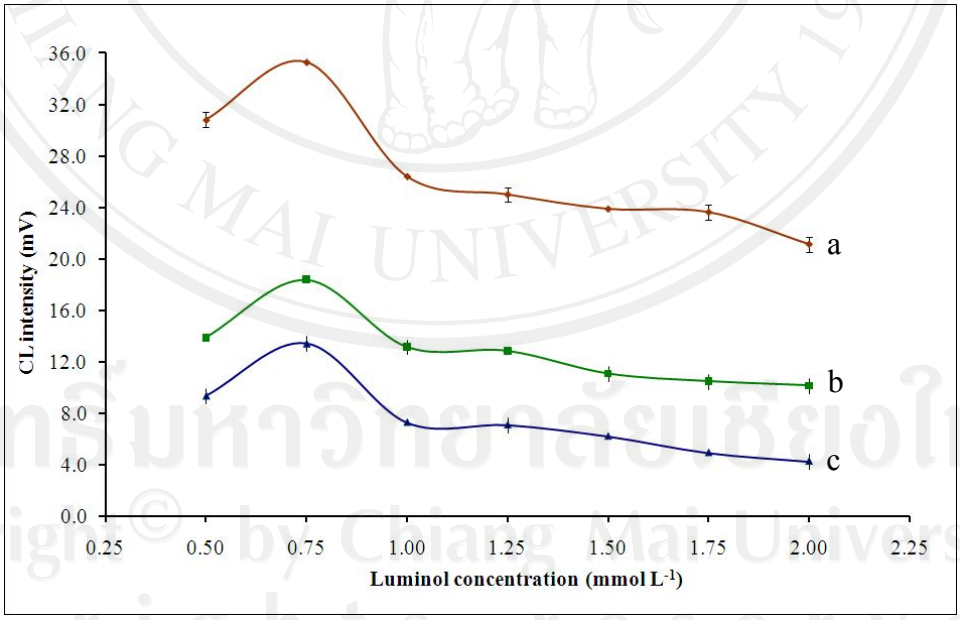

Figure 3.8 Effect of luminol concentration on the CL intensity of NFZ (a), NFT (b) and FZD (c)

3.3.1.5 Effect of sodium hydroxide concentration on the CL intensity

Although the CL signal could also be observed in alkaline medium, the strong CL signal was obtained with sodium hydroxide (NaOH) [63]. The effect of NaOH concentration on the CL reaction was shown in Figure 3.9 and Table 3.8. The maximum CL intensity was obtained when 0.4 mol L^{-1} NaOH was used. Lower or higher concentration of NaOH caused a decrease in CL signal. Therefore, 0.4 mol L^{-1} NaOH was employed.

Table 3.8 Effect of sodium hydroxide concentration on the CL intensity of NFZ, NFT and FZD

Sodium hydroxide concentration	**CL intensity (mV)* ± S.D.**		
(mol L^{-1})	**NFZ**	**NFT**	**FZD**
0.30	44.9 ± 0.6	16.4 ± 0.0	9.6 ± 0.0
0.35	46.3 ± 0.0	17.3 ± 0.6	12.4 ± 0.6
0.40	58.1 ± 0.0	28.8 ± 0.0	19.2 ± 0.0
0.45	50.2 ± 0.0	22.0 ± 0.0	11.6 ± 0.0
0.50	49.9 ± 0.6	17.1 ± 0.0	11.8 ± 0.0
0.55	46.0 ± 0.6	17.3 ± 0.6	10.7 ± 0.6
0.60	48.1 ± 0.6	18.5 ± 0.0	9.5 ± 0.0

*average of triplicate results

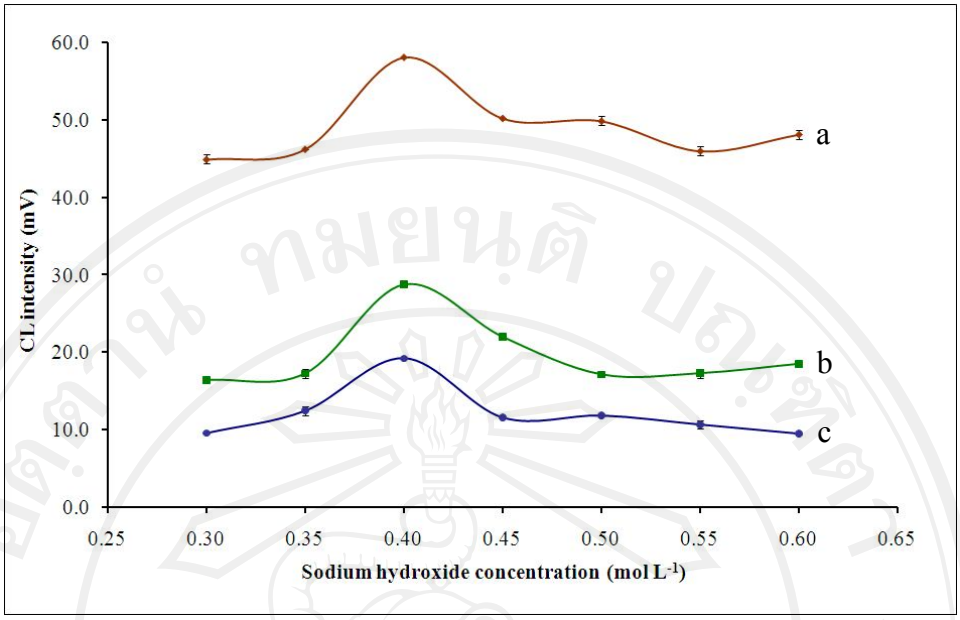

Figure 3.9 Effect of sodium hydroxide concentration on the CL intensity of NFZ (a), NFT (b) and FZD (c)

3.3.1.6 Effect of potassium ferrocyanide concentration on the CL intensity

Luminol (5-aminophthalylhydrazide) is so far the most frequently used CL reagent. The CL emission of luminol is based on its oxidation by hydrogen peroxide, hexacyanoferrate(III), permanganate, *N*-bromosuccinimide (or *N*-chlorosuccinimide), periodate, dichromate, persulphate, dichlorocyanurate or trichlorocyanuric acid, chlorate and electrogenerated hypobromite in alkaline medium. According to the well-known mechanism the supposed emitter is excited 3-aminophthalate anion whose maximum emission occurs at 425 nm [64].

Some catalysts such as Cu(II), Co(II) and Fe(III)/Fe(II) were implemented into the luminol/oxidant system to improve sensitivity of the indirect assay [64]. Thus in this study Fe(II) was selected as a catalyst in the determination of nitrofurans. The CL reaction can be catalyzed by potassium ferocyanide ($K_4Fe(CN)_6$) and it can also

oxidize luminol to generate strong CL in alkaline solution. The 40 to 80 μmol L^{-1} $K_4Fe(CN)_6$ (Figure 3.10 and Table 3.9) was examined. The maximum CL intensity was obtained at 50 μmol L^{-1} $K_4Fe(CN)_6$ and was chosen for consequent experiments.

Table 3.9 Effect of potassium ferrocyanide concentration on the CL intensity of NFZ, NFT and FZD

$K_4Fe(CN)_6$ concentration	**CL intensity (mV)* ± S.D.**		
(μmol L^{-1})	**NFZ**	**NFT**	**FZD**
40	171.3 ± 0.6	75.7 ± 0.6	28.0 ± 0.0
50	195.0 ± 0.6	100.0 ± 0.6	50.7 ± 0.0
60	165.0 ± 0.6	72.7 ± 0.6	28.7 ± 0.0
70	160.3 ± 0.6	69.0 ± 0.0	27.0 ± 0.0
80	155.3 ± 0.6	65.0 ± 0.0	27.3 ± 0.0

*average of triplicate results

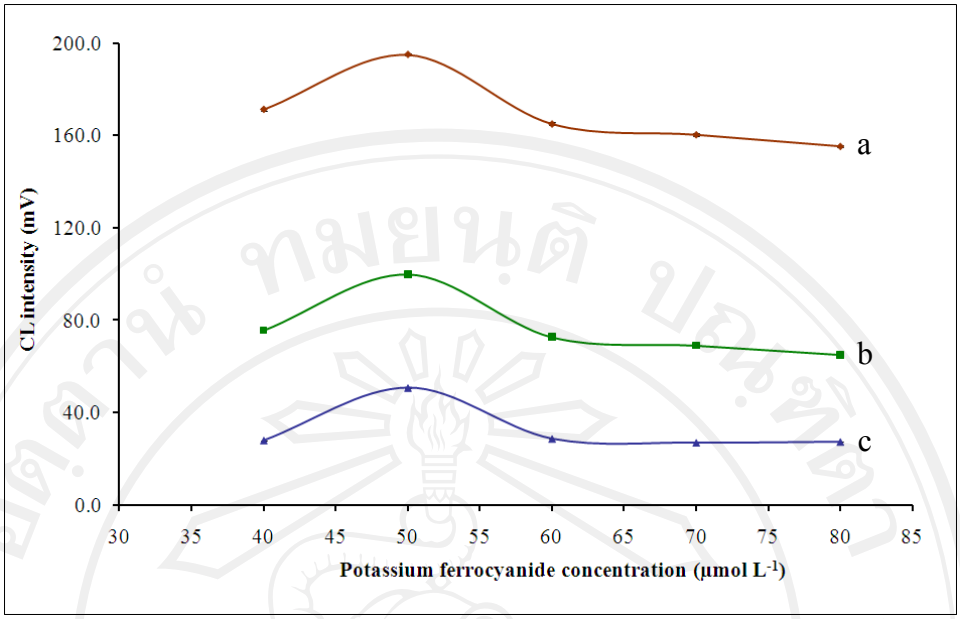

Figure 3.10 Effect of potassium ferrocyanide concentration on the CL intensity of NFZ (a), NFT (b) and FZD (c)

3.3.1.7 Effect of hydrogen peroxide concentration on the CL intensity

The dependence of the CL intensity on H_2O_2 was examined in the 0.01 to 1.10 mol L^{-1} concentration range. H_2O_2 was mixed with 0.1 % (w/v) SHMP solution in deionized water and being used as an oxidant/surfactant stream. The maximum CL intensity can be observed when the H_2O_2 concentration was 0.10 mol L^{-1} (Figure 3.11 and Table 3.10). Therefore, 0.10 mol L^{-1} of H_2O_2 was chosen as the optimum H_2O_2 concentration.

Table 3.10 Effect of hydrogen peroxide concentration on CL the intensity of NFZ, NFT and FZD

H_2O_2concentration (mol L^{-1})	CL intensity (mV)* ± S.D.		
	NFZ	NFT	FZD
0.01	6.8 ± 1.2	12.1 ± 0.6	15.0 ± 0.6
0.05	18.2 ± 0.6	15.3 ± 0.6	18.7 ± 0.6
0.10	22.0 ± 0.0	17.1 ± 0.6	20.3 ± 0.6
0.30	18.6 ± 0.0	13.0 ± 0.0	14.3 ± 0.0
0.50	16.1 ± 0.6	11.6 ± 0.6	10.5 ± 0.6
0.70	15.4 ± 0.6	10.8 ± 0.6	9.3 ± 0.0
0.90	10.9 ± 0.6	10.6 ± 0.6	7.4 ± 1.0
1.10	8.4 ± 1.0	7.5 ± 0.6	6.6 ± 0.6

*average of triplicate results

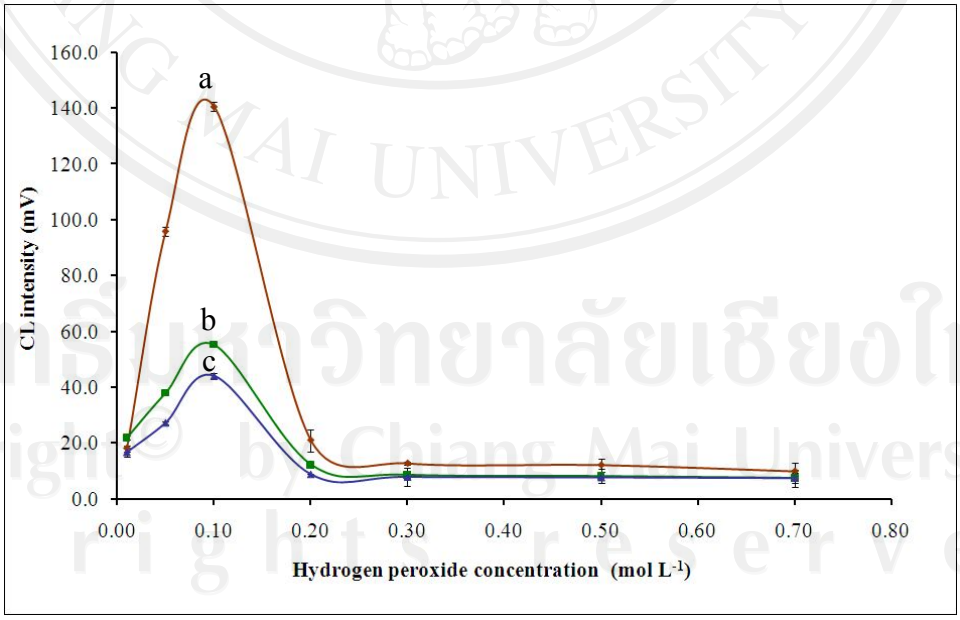

Figure 3.11 Effect of hydrogen peroxide concentration on CL the intensity of NFZ (a), NFT (b) and FZD (c)

3.3.1.8 Effect of injected sample volume on the CL intensity

It is necessary to optimize the injected sample volume to achieve the desired sensitivity with appropriate sample throughput. Since the amounts of sample injected into the microfluidic system should be sufficient to permit effective CL reaction. An increase in sample volume normally leads to an increase of the emitted CL signal [62]. The influence of the injected sample volume was assessed for values ranging from 10 to 500 µL (Figure 3.12 and Table 3.11 – 3.12). With an injection value of 10 µL, the lowest CL intensity was obtained. With volumes greater than 100 µL, the variation in the CL intensity was of minor significance. Therefore, an injection volume of 100 µL was selected since it compromised a good sensitivity, reproducibility and reasonable sample throughput.

Table 3.11 Effect of injection volume on the CL intensity

Aspiration volume of sample (µL)	CL intensity (mV)* ± S.D.		
	NFZ	NFT	FZD
10	33.3 ± 1.2	13.0 ± 0.0	12.3 ± 0.6
50	91.3 ± 1.5	38.3± 0.6	31.3 ± 0.6
75	140.7 ± 3.8	52.7 ± 0.6	45.0 ± 0.0
100	209.7 ± 2.5	86.0 ± 0.0	70.3 ± 1.2
125	237.0 ± 0.0	96.3 ± 1.5	77.3 ± 0.6
150	255.0 ± 3.0	105.3 ± 1.2	83.0 ± 0.0

*average of triplicate results

Table 3.11 Continued

Aspiration volume of sample (µL)	CL intensity (mV)* ± S.D.		
	NFZ	NFT	FZD
200	278.7 ± 0.6	110.7 ± 0.6	89.0 ± 2.0
250	299.3 ± 4.2	121.0 ± 1.0	96.7 ± 0.6
300	306.7 ± 2.5	125.3 ± 0.6	102.3 ± 1.2
350	318.0 ± 2.6	129.7 ± 0.6	106.0 ± 0.0
500	328.0 ± 1.7	144.7 ± 5.9	109.7 ± 0.6

*average of triplicate results

Table 3.12 Sample throughput of injection volume on the CL intensity

Aspiration volume of sample	Sample throughput (h^{-1})* ± S.D.		
(µL)	NFZ	NFT	FZD
10	67.9 ± 0.0	83.7 ± 6.5	85.0 ± 6.1
50	44.1 ± 0.3	63.5 ± 0.7	55.7 ± 2.0
75	39.6 ± 1.1	50.2 ± 0.8	44.8 ± 2.2
100	33.5 ± 0.5	40.1 ± 0.7	37.8 ± 1.0
125	31.0 ± 1.0	36.9 ± 1.1	35.1 ± 0.8
150	30.8 ± 1.0	33.2 ± 1.2	32.4 ± 1.7

Table 3.12 Continued

Aspiration volume of sample (µL)	Sample throughput (h^{-1})* ± S.D.		
	NFZ	NFT	FZD
200	26.7 ± 1.0	29.3 ± 0.4	30.7 ± 0.5
250	21.7 ± 0.6	25.2 ± 0.4	27.0 ± 0.8
300	20.3 ± 0.2	23.9 ± 2.1	23.6 ± 0.6
350	19.6 ± 0.9	22.1 ± 0.2	22.9 ± 1.0
500	16.2 ± 0.8	18.5 ± 1.5	19.3 ± 0.3

*average of triplicate results

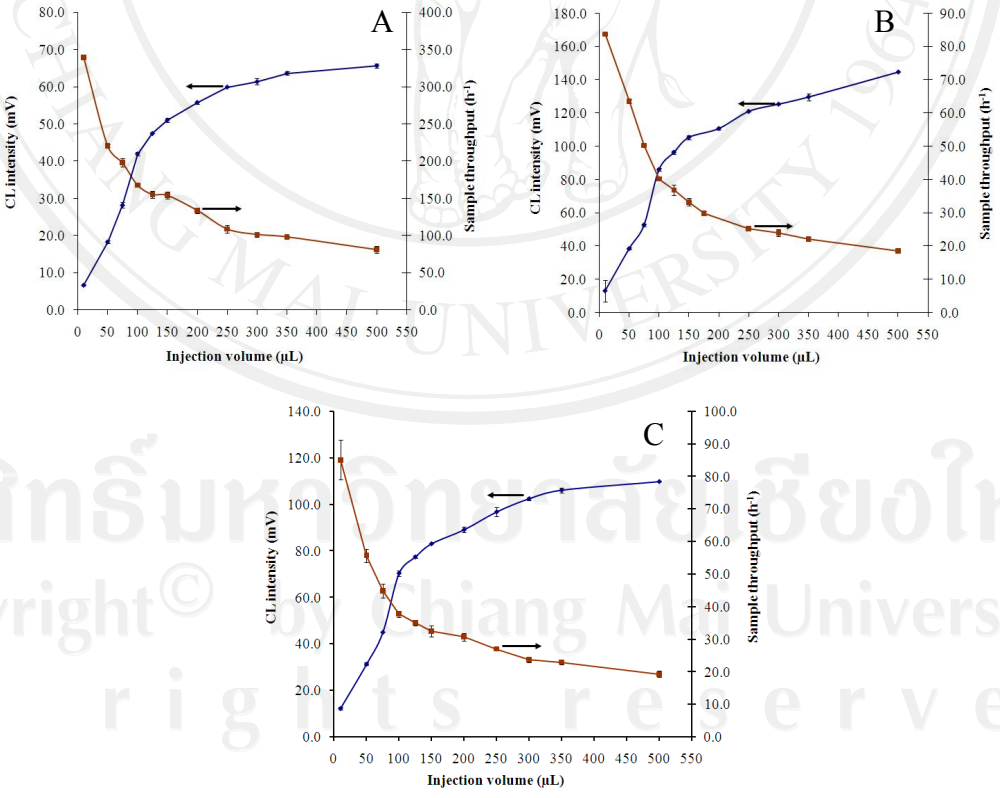

Figure 3.12 Effect of injection volume on the CL intensity (blue line) and sample throughput (red line) for the determination of NFZ (A), NFT (B) and FZD (C)

3.3.1.9 Effect of flow rate on the CL intensity

The flow rate is another important parameter to transport fluids on microfluidic systems as the time taken to transfer the excited product into the microfluidic device is critical for maximum collection of the emitted light as reported previously [28]. The flow rate is conveniently controlled by the peristaltic pump. Effect of flow rate of a carrier stream, reagent streams and a stream of oxidant/surfactant solution were studied, by determination of concentration of standard NFZ, NFT and FZD solutions which were flowed into the microfluidic chemiluminescence manifold as shown in Figure 2.5. The flow rates were varied from 0.05 to 0.40 mL min^{-1}. The effect of flow rate was evaluated by compromising between the CL intensity versus sample throughput. The results were shown in Figure 3.13 - 3.15 and Table 3.13 - 3.15. It was found that the CL intensity increased with the carrier stream (C) the flow rate up to 0.15 mL min^{-1} and remained almost constant above this value. The CL intensity increased with oxidant/surfactant stream (R1) the flow rate up to 0.15 mL min^{-1}; increasing the flow rate above this point causes the CL intensity to diminish gradually due to the poorer chemiluminescence reactivity occurring in the reaction zone of the microchip. The effect of flow rate of reagent stream (R2) on both systems sensitivity and sampling rate were studied. As expected, at lower flow rates higher sensitivity but lower sample throughput was obtained (Figure 3.15). Consequently, a flow rate of 0.15 mL min^{-1} was chosen as a compromise between the conflicting requirements for high sensitivity and high sample throughput. In addition, it indicates that the chemiluminescence reactivity is not completed, and the extent of the reaction clearly increases with an increase of a residence time of the injection plug inside the microfluidic devices. Therefore, the optimum flow rate of all stream (carrier

stream, reagent streams and a oxidant/surfactant stream) were 0.15 mL min^{-1}. This value was selected as a compromise situation between the sensitivity and the sampling rate obtained.

Table 3.13 Effect of flow rate of a carrier stream on the CL intensity

Flow rate (mL min^{-1})	**CL intensity (mV)* ± S.D.**		
	NFZ	**NFT**	**FZD**
0.05	160.3 ± 2.3	66.7 ± 1.2	60.0 ± 1.7
0.10	232.7 ± 4.0	94.3 ± 1.5	84.3 ± 0.6
0.15	259.7 ± 2.3	105.3 ± 1.5	95.0 ± 1.0
0.20	265.0 ± 2.0	109.7 ± 1.5	98.3 ± 1.2
0.25	268.7 ± 1.2	111.0 ± 1.0	99.0 ± 1.7
0.30	262.3 ± 3.8	112.0 ± 1.0	98.0 ± 0.0
0.35	257.7 ± 2.5	113.0 ± 1.0	100.7 ± 1.5
0.40	252.3 ± 2.5	113.3 ± 1.5	102.7 ± 0.6

*average of triplicate results

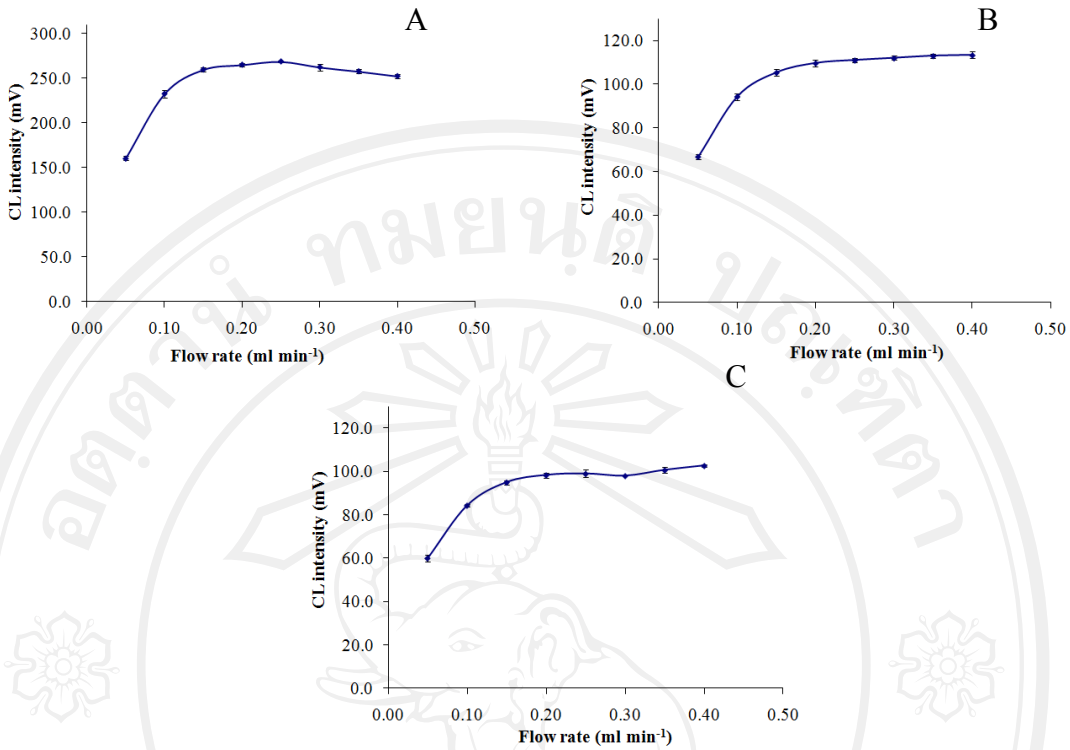

Figure 3.13 Effect of flow rate of a carrier stream on the CL intensity for the determination of NFZ (A), NFT (B) and FZD (C)

Table 3.14 Effect of flow rate of a oxidant/surfactant stream on the CL intensity

Flow rate (mL min^{-1})	CL intensity (mV)* ± S.D.		
	NFZ	NFT	FZD
0.05	213.3 ± 2.3	88.0 ± 1.0	77.3 ± 0.6
0.10	279.7 ± 3.1	111.7 ± 1.2	104.0 ± 1.0
0.15	302.0 ± 3.6	120.0 ± 0.0	114.7 ± 2.5
0.20	289.7 ± 2.3	121.3 ± 0.6	114.3 ± 1.5
0.25	271.3 ± 1.5	119.3 ± 1.2	115.7 ± 0.6
0.30	258.0 ± 0.0	113.3 ± 0.6	112.0 ± 1.0
0.35	241.0 ± 1.0	105.7 ± 0.6	107.0 ± 0.0
0.40	221.7 ± 2.5	97.3 ± 0.6	100.3 ± 1.2

*average of triplicate results

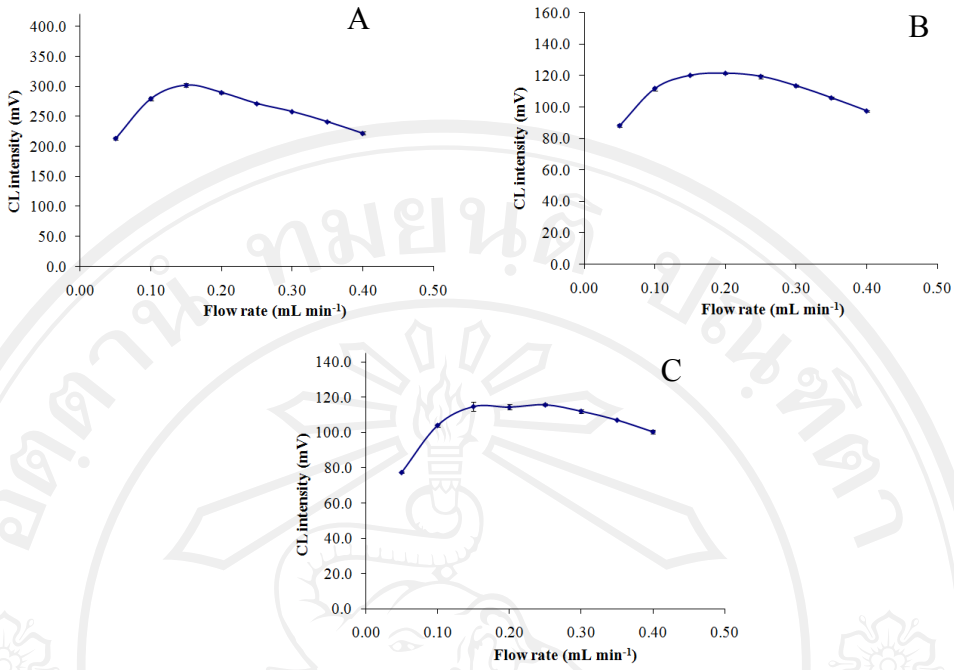

Figure 3.14 Effect of flow rate of a oxidant/surfactant stream on the CL intensity for the determination of NFZ (A), NFT (B) and FZD (C)

Table 3.15 Effect of flow rate of a reagent stream on the CL intensity

Flow rate (mL min^{-1})	CL intensity (mV)* ± S.D.		
	NFZ	NFT	FZD
0.05	183.3± 4.0	98.3± 1.2	112.7± 3.1
0.10	227.3± 0.6	89.7± 0.6	98.3± 0.6
0.15	230.7± 0.6	87.0± 0.0	91.3± 0.6
0.20	209.0± 2.0	75.0± 1.0	74.0± 1.0
0.25	174.0± 1.7	62.0± 0.0	60.7± 1.2
0.30	146.3± 1.2	51.0± 1.0	49.0± 0.0
0.35	125.7± 0.6	43.7± 0.6	40.7± 0.6
0.40	110.0± 0.0	38.0± 1.0	35.0± 0.0

*average of triplicate results

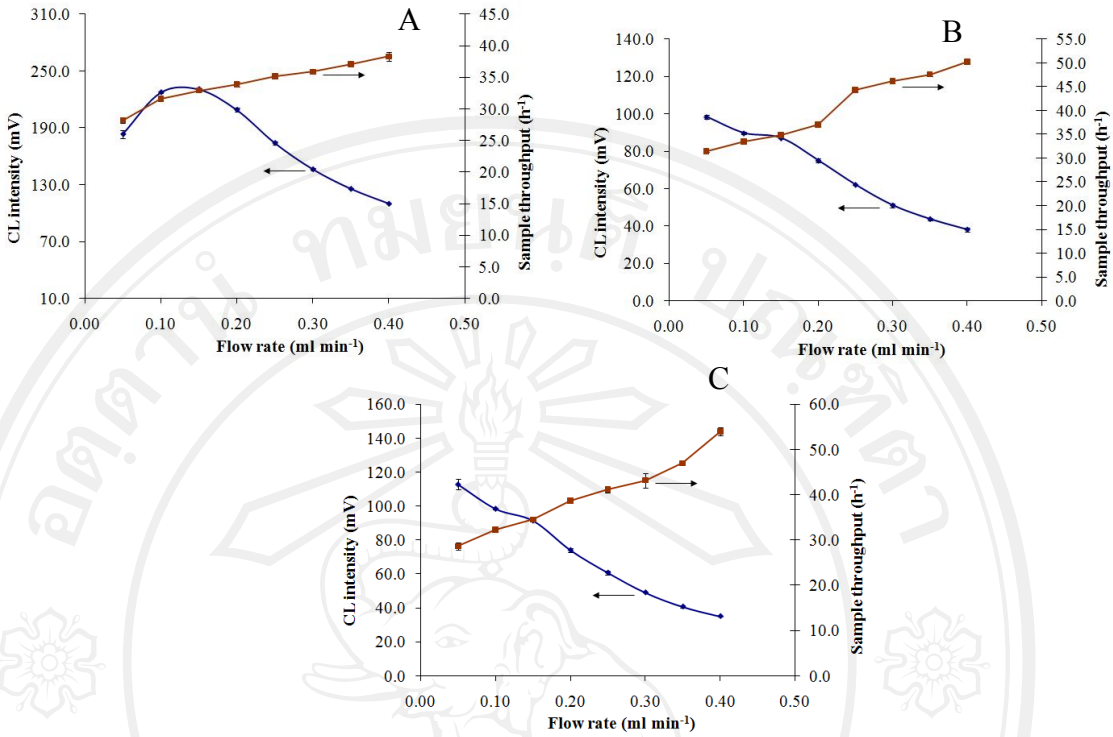

Figure 3.15 Effect of flow rate of a reagent stream on the CL intensity (blue line) and sample throughput (red line) for the determination of NFZ (A), NFT (B) and FZD (C)

3.3.2 Summary of the studied range and optimum conditions

A three-line flow diagram of the proposed microfluidic chemiluminescence manifold was displayed as in Figure 2.5. Table 3.16 shown the ranges over which the variables involved in the microfluidic chemiluminescence system and their optimum values. The method gives a steady signal-to-noise ratio, high sensitivity, fast analysis and reducing the reagent consumption with minimum waste production.

Table 3.16 Optimized operating conditions for the determination of nitrofurans by use of the microfluidic chemiluminescence system

Parameters	Range studied	Optimal values
PMT voltage (V)	750 - 1000	900
$K_4Fe(CN)_6$ (μmol L^{-1})	10 - 80	50
Luminol (mmol L^{-1})	0.50 - 2.00	0.75
H_2O_2 (mol L^{-1})	0.01 - 0.70	0.10
NaOH (mol L^{-1})	0.30 - 0.60	0.40
SHMP (% w/v)	0.05 - 0.50	0.10
Injection volume (μL)	10 - 500	100
Flow rate of a carrier stream (mL min^{-1})	0.05 - 0.40	0.35
Flow rate of a reagent stream (mL min^{-1})	0.05 - 0.40	0.15
Flow rate of oxidant/surfactant stream (mL min^{-1})	0.05 - 0.40	0.15

3.3.3 Analytical characteristics of merit for determining nitrofurans

3.3.3.1 Linearity and calibration curve

Linearity of the standard solution of NFZ, NFT and FZD concentration from 0.10 to 23.00 mg L^{-1} was studied by injection of each standard solution of NFZ, NFT and FZD into the microfluidic chemiluminescence system under the suitable experimental conditions as depicted in Table 3.16. The linear range of the calibration graphs were obtained for NFZ, NFT and FZD standards at the concentration ranging

from 0.30 – 9.00 mg L^{-1} nitrofurans. The results obtained were shown in Table 3.17 and Figure 3.16. All measurements were made in triplicate injections.

Table 3.17 The CL intensity from various nitrofurans standards at the concentration ranging from 0.10 – 23.00 mg L^{-1} nitrofurans

Nitrofurans concentration (mg L^{-1})	**CL intensity (mV)* ± S.D.**		
	NFZ	**NFT**	**FZD**
0.10	5.3 ± 0.6	-	-
0.30	14.7 ± 0.6	6.7 ± 0.6	7.0 ± 0.0
0.50	27.3 ± 0.6	9.0 ± 0.0	13.0 ± 0.0
1.00	49.7 ± 0.6	23.7 ± 0.6	25.0 ± 0.0
1.50	76.0 ± 1.0	36.7 ± 0.6	33.0 ± 0.0
2.00	104.0 ± 0.0	50.0 ± 0.0	43.3 ± 1.2
2.50	125.0 ± 3.0	65.3 ± 0.6	53.0 ± 1.0
3.00	167.0 ± 2.6	76.7 ± 0.6	65.3 ± 0.6
3.50	186.3 ± 2.1	100.0 ± 1.0	75.3 ± 0.6
4.00	210.7 ± 0.6	112.3 ± 1.2	87.3 ± 0.6
4.50	231.3 ± 2.3	127.0 ± 0.0	97.3 ± 0.6
5.00	262.0 ± 0.0	140.0 ± 0.6	105.0 ± 0.0
6.00	306.7 ± 2.1	164.3 ± 1.2	123.7 ± 0.6
7.00	366.3 ± 2.1	194.3 ± 2.1	142.0 ± 0.6
9.00	478.3 ± 1.5	253.7 ± 1.2	184.7 ± 2.3
10.00	535.3 ± 2.3	285.0 ± 1.0	-
11.00	586.3 ± 3.2	306.7 ± 1.5	-
13.00	707.0 ± 2.6	-	-

*average of triplicate results

Table 3.17 Continued

Nitrofurans concentration (mg L^{-1})	CL intensity (mV)* ± S.D.		
	NFZ	NFT	FZD
15.00	806.0 ± 2.6	-	-
17.00	923.0 ± 2.6	-	-
20.00	1063.7 ± 2.5	-	-
23.00	1228.3 ± 1.5	-	-

*average of triplicate results

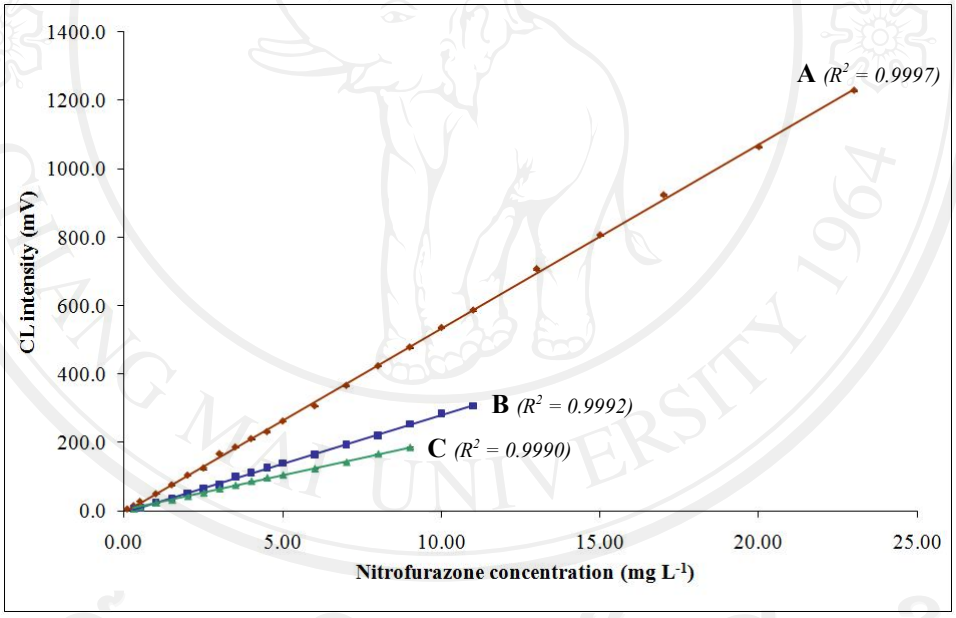

Figure 3.16 Linear calibration graphs obtained under the optimal conditions for determination of NFZ (A), NFT (B) and FZD (C)

3.3.3.2 Detection and quantification limits

Under the optimum conditions for the determination of nitrofurans: nitrofurazone (NFZ), nitrofurantoin (NFT) and furazolidone (FZD). The detection limit (S/N = 3) of the method was 0.058, 0.11 and 0.12 mg L^{-1} with a quantification limit (S/N = 10) of 0.19, 0.36 and 0.40 mg L^{-1} respectively [57], which are better than those previous by reported by Thongsrisomboon et al. [46].

3.3.3.3 Precision

The precision of the proposed method was verified by 15 replicated determinations of 2.0 mg L^{-1} standard nitrofurans (Figure 3.17), under the optimum conditions. The relative standard deviation was found to be 1.9, 0.8 and 1.0 % for NFZ, NFT and FZD, respectively (Table 3.18).

3.3.3.4 Intra-day and inter-day variations

The intra-day variations of the method were determined using triplicate injections of standard nitrofurans solution and analysed on the same day. Inter-day precision was studied by comparing the results of assays performed on different days on the same injected standard nitrofurans solution in three replicates. The result was expressed in term of percentage relative standard deviation (% RSD). Results were shown in Table 3.19 - 3.20, which are similar to those previous by reported by Thongsrisomboon et al. [46].

Table 3.18 Replicate measurements by using standard 2.0 mg L^{-1} nitrofurans

Experimental number	CL intensity (mV)		
	NFZ	**NFT**	**FZD**
1	106.0	54.0	38.0
2	102.0	54.0	38.0
3	105.0	53.0	40.0
4	105.0	54.0	40.0
5	106.0	53.0	39.0
6	104.0	54.0	38.0
7	106.0	53.0	39.0
8	104.0	54.0	38.0
9	105.0	52.0	38.0
10	109.0	53.0	38.0
11	108.0	52.0	39.0
12	105.0	52.0	40.0
13	108.0	53.0	41.0
14	108.0	54.0	39.0
15	107.0	54.0	40.0
$\overline{x}$	105.9	53.3	39.0
S.D.	1.9	0.8	1.0
% RSD	1.8	1.5	2.6

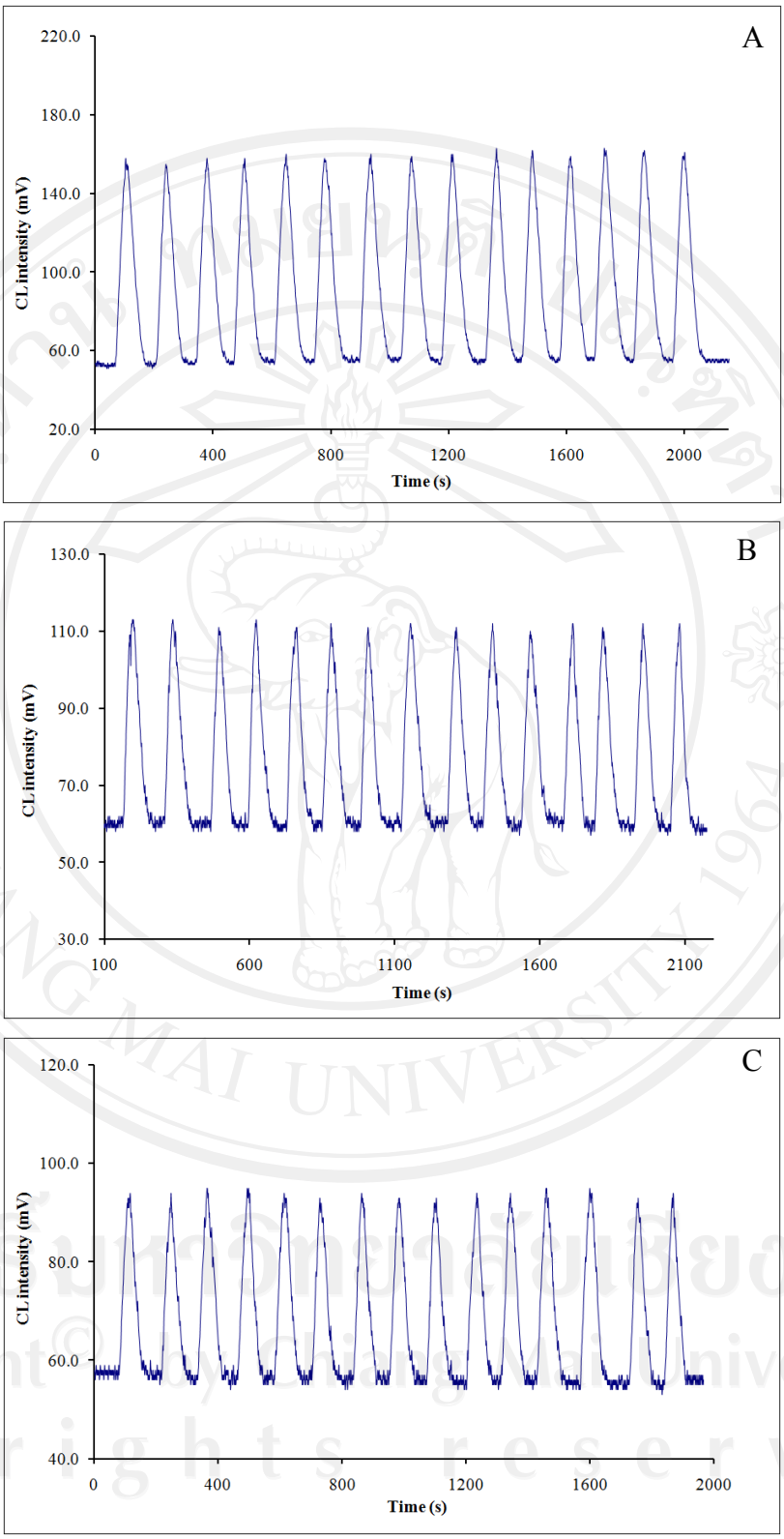

Figure 3.17 Repeatability in the measurements obtained with the proposed method for determination of NFZ (A), NFT (B) and FZD (C)

Table 3.19 Intra-day variations

Times (hour)	CL intensity (mV)*		
	NFZ	**NFT**	**FZD**
3	108.44	52.89	41.44
6	111.33	50.44	43.00
9	110.56	53.67	41.33
Mean	110.11	52.33	41.93
S.D.	1.49	1.68	0.93
% RSD	1.36	3.21	2.22

*average of triplicate results

Table 3.20 Inter-day variations

Times (Day)	CL intensity (mV)*		
	NFZ	**NFT**	**FZD**
1	107.33	65.00	43.33
2	110.67	64.33	44.00
3	113.67	61.67	41.67
Mean	110.56	63.67	43.00
S.D.	3.17	1.76	1.20
% RSD	2.87	2.77	2.91

*average of triplicate results

Table 3.21 Analytical figures of merit for determining nitrofurans

Analytical parameters	NFZ	NFT	FZD
Regression equation [a]	*I = 53.764C -3.894*	*I = 28.551C - 4.5994*	*I = 20.222C + 3.4745*
Correlation coefficient (r^2)	0.9997	0.9992	0.9990
Detection limit (mg L^{-1}) [b]	0.058	0.11	0.12
Quantification limit (mg L^{-1})	0.19	0.36	0.40
% Relative standard deviation (RSD)	1.80	1.50	2.60
Intra-day precisions (%RSD)	1.36	3.21	2.22
Inter-day precisions (%RSD)	2.87	2.77	2.91

[a] *I* = analytical signal in mV and *C* = concentration of each nitrofurans (mg L^{-1})

[b] The limit of detection, calculated at three times the standard deviation of the noise

3.3.4 Interference studies

The possible interferences of common excipients (Acacia, Bentonite, Carboxyl methyl cellulose, Fructose, Glucose, Lactose, Methyl cellulose, Polyethylene glycol, Starch, Sucrose), some cations (Al^{3+}, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) and anions (Cl^{-}, CO_3^{2-}, SO_4^{2-}, NO_3^{-}, HPO_4^{2-},), which might be used in pharmaceutical preparations and animal feeds samples, were investigated. The investigation was carried out by injecting each 2 mg L^{-1} nitrofuran standard solution containing certain foreign compounds, which are common additives in nitrofurans preparations. Weight ratios of excipients, cations and anions: analyte, ranging from 20 – 2,000, were injected into the microfluidic chemiluminescence system (Table 3.22 - 3.23). The maximum tolerable concentrations for each excipient and common ions are shown in

Table 3.24 - 3.25. A substance was considered not to interfere if it caused a relative error of less than 5% for 2 mg L^{-1} of nitrofurans. It can be seen that all interferences showed no serious effect on the determination of nitrofurans even though they are present at 20 to 2,000 times the weight ratio of nitrofurans.

Table 3.22 Interference studies of common excipients for 2.0 mg L^{-1} nitrofurans by proposed method

Interferences	**Nitrifurans-to-interference weight ratio**	**% Relative error***		
		NFZ	**NFT**	**FZD**
Glucose	2 : 0	-	-	-
	2 : 2	0.04	-25.07	0.00
	2 : 20	5.92	-15.50	-0.68
	2 : 200	9.80	-1.74	5.44
	2 : 2000	17.45	14.42	1.36
Sucrose	2 : 0	-	-	-
	2 : 2	-0.31	5.98	12.24
	2 : 20	-2.75	7.30	2.72
	2 : 200	4.56	9.27	10.88
	2 : 2000	26.85	40.17	38.10
Fructose	2 : 0	-	-	-
	2 : 2	1.73	12.32	-4.10
	2 : 20	5.63	7.97	1.16
	2 : 200	14.29	27.54	20.08
	2 : 2000	46.32	66.67	44.27

*average of triplicate results

Table 3.22 Continued

Interferences	Nitrifurans-to-interference weight ratio	% Relative error*		
		NFZ	NFT	FZD
Lactose	2 : 0	-	-	-
	2 : 2	-0.99	-0.06	-4.04
	2 : 20	1.06	-12.81	-17.73
	2 : 200	-4.40	-25.56	-10.20
	2 : 2000	-37.15	-84.83	-77.28
Strach	2 : 0	-	-	-
	2 : 2	3.83	6.54	-4.10
	2 : 20	6.51	7.19	6.41
	2 : 200	0.38	0.65	-11.46
	2 : 2000	-9.58	-54.25	-64.04
Bentonite	2 : 0	-	-	-
	2 : 2	-16.48	21.84	-3.93
	2 : 20	-4.83	8.62	-6.24
	2 : 200	-26.41	2.87	7.62
	2 : 2000	-86.37	-46.55	-17.78
Methyl cellulose	2 : 0	-	-	-
	2 : 2	-3.41	-5.37	-3.42
	2 : 20	-5.83	5.24	-14.53
	2 : 200	-31.33	-23.92	-58.12
	2 : 2000	-302.96	-482.50	-618.80

*average of triplicate results

Table 3.22 Continued

Interferences	Nitrifurans-to-interference weight ratio	% Relative error*		
		NFZ	NFT	FZD
Acacia	2 : 0	-	-	-
	2 : 2	-3.48	8.28	-5.13
	2 : 20	0.58	2.81	-18.80
	2 : 200	-2.32	-26.63	-57.26
	2 : 2000	-200.3	-535.2	-687.2
Sodium carboxymethyl cellulose	2 : 0	-	-	-
	2 : 2	-0.85	-0.06	15.05
	2 : 20	3.55	4.40	18.65
	2 : 200	9.70	16.51	25.85
	2 : 2000	-1.14	-15.36	-15.19
Propylene glycol	2 : 0	-	-	-
	2 : 2	1.72	-12.00	7.32
	2 : 20	4.60	-2.67	10.31
	2 : 200	3.45	-8.00	4.33
	2 : 2000	-5.17	-32.00	-9.12

*average of triplicate results

Table 3.23 Interference studies of some common ions for 2.0 mg L^{-1} nitrofurans by proposed method

Interferences	Nitrifurans-to-interference weight ratio	% Relative error*		
		NFZ	NFT	FZD
K^+ (KCl)	2 : 0	-	-	-
	2 : 2	2.87	-2.82	-2.03
	2 : 20	-1.44	-12.77	-6.92
	2 : 200	-17.82	-33.78	-25.09
	2 : 2000	-32.47	-76.89	-76.10
Na^+ (NaCl)	2 : 0	-	-	-
	2 : 2	-3.17	6.41	1.28
	2 : 20	-3.17	-5.15	-7.69
	2 : 200	-10.05	-20.93	-28.21
	2 : 2000	-76.19	-131.34	-151.28
Ca^{2+} ($CaCl_2.2H_2O$)	2 : 0	-	-	-
	2 : 2	-18.81	-34.12	-38.92
	2 : 20	-26.77	-49.95	-61.80
	2 : 200	-36.56	-67.89	-79.81
	2 : 2000	-57.34	-80.21	-89.43
Mg^{2+} ($MgCl_2.6H_2O$)	2 : 0	-	-	-
	2 : 2	-11.84	-9.23	-17.78
	2 : 20	22.03	19.49	42.26
	2 : 200	80.74	75.90	65.74
	2 : 2000	115.64	98.67	82.25

*average of triplicate results

Table 3.23 Continued

Interferences	Nitrifurans-to-interference weight ratio	% Relative error*		
		NFZ	NFT	FZD
Cl^- (NaCl)	2 : 0	-	-	-
	2 : 2	-3.28	-0.57	-3.79
	2 : 20	-5.09	-5.24	-0.76
	2 : 200	-10.21	-16.12	-21.21
	2 : 2000	-46.64	-66.41	-98.48
CO_3^{2-} (Na_2CO_3)	2 : 0	-	-	-
	2 : 2	9.12	9.78	10.08
	2 : 20	26.41	40.03	22.80
	2 : 200	23.78	46.52	54.34
	2 : 2000	28.17	60.75	78.98
SO_4^{2-} (Na_2SO_4)	2 : 0	-	-	-
	2 : 2	1.73	-4.82	-12.76
	2 : 20	-2.28	-4.82	-14.87
	2 : 200	-13.03	-24.56	-42.35
	2 : 2000	-50.79	-82.02	-57.46
NO_3^- ($NaNO_3$)	2 : 0	-	-	-
	2 : 2	-0.72	1.10	-4.66
	2 : 20	-2.22	-11.98	-5.18
	2 : 200	-5.46	-19.83	-17.74
	2 : 2000	-34.88	-25.62	-30.21

*average of triplicate results

Table 3.23 Continued

Interferences	Nitrifurans-to-interference weight ratio	% Relative error*		
		NFZ	NFT	FZD
$Al^{3+}(Al(NO_3)_3).9H_2O)$	2 : 0	-	-	-
	2 : 2	-48.99	-174.00	-177.78
	2 : 20	-93.91	-355.24	-366.67
	2 : 200	-115.32	-400.34	-450.43
	2 : 2000	-157.45	-489.90	-500.14
HPO_4^{2-} (Na_2HPO_4)	2 : 0	-	-	-
	2 : 2	11.20	12.81	26.28
	2 : 20	17.21	23.54	30.77
	2 : 200	11.75	10.13	21.79
	2 : 2000	15.65	18.56	23.43

*average of triplicate results

Table 3.24 Maximum tolerable concentration for the determination of 2.0 mg L^{-1} nitrofurans in the presence of common excipients

Common excipients	Tolerance (mg L^{-1})*		
	NFZ	NFT	FZD
Glucose	20	2	200
Sucrose	2000	2	2
Fructose	20	2	200
Lactose	2000	20	20
Strach	20	2	20
Bentonite	2	2	20
Methyl cellulose	20	2	20
Acacia	2000	200	20
Sodium carboxymethyl cellulose	200	200	2
Propylene glycol	2000	200	2

*average of triplicate results

Table 3.25 Maximum tolerable concentration for the determination of 2.0 mg L^{-1} nitrofurans in the presence of some common ions

Common ions	Tolerance (mg L^{-1})*		
	NFZ	NFT	FZD
K^+ (KCl)	200	20	20
Na^+ (NaCl)	200	2	20
Ca^{2+} ($CaCl_2.2H_2O$)	2	2	2
Mg^{2+} ($MgCl_2.6H_2O$)	2	2	2
Cl^- (NaCl)	200	200	200
CO_3^{2-} (Na_2CO_3)	2	2	2
SO_4^{2-} (Na_2SO_4)	200	200	2
NO_3^- ($NaNO_3$)	200	20	200
Al^{3+} ($Al(NO_3)_3.9H_2O$)	2	2	2
HPO_4^{2-} (Na_2HPO_4)	2	2	2

*average of triplicate results

3.3.5 Real samples determination

The proposed microfluidic chemiluminescence method was applied to determine nitrofurans in pharmaceutical preparations and animal feeds samples. The present microfluidic chemiluminescence system was employed for nitrofurans determination in 10 different real samples. Two samples of pure nitrofuran tablets, one sample of blend nitrofuran tablets, one sample of nitrofuran ointment and five animal feeds (poultry and porcine) were analyzed by the proposed microfluidic chemiluminescence procedure under optimum experimental conditions after appropriate sample pretreatments. Table 3.26 shows the analytical data related to the analysis of six samples of pharmaceutical preparations together with another four animal feeds samples. The results ware compared with those obtained by reference method (official BP or HPLC method). Evaluation of the proposed method was also carried out by comparison the results obtained by reference method using student *t*-test, with 4 degree of freedom so the critical value of *t*-test is 2.776 at confidence interval of 95%. The observed value of *t*-value is less than the critical value so the both methods were in good agreement. It may thus be concluded that there are no significant differences between the proposed method and the reference procedure.

Table 3.26 Determination of nitrofurans in pharmaceutical and animal feeds samples

Sample	Extract procedure	Amount found (mean ± SD[c])			± t_{value}[f]
		μFIA-CL	BP method[d]	HPLC[e]	
NFZ cream	Nil.	166.04±9.87 mg kg^{-1}	170.37 ± 6.17 mg L^{-1}	-	1.87
NFT tablets	Nil.	91.56 ± 4.02 mg tab^{-1}	94.29 ± 0.62 mg tab^{-1}	-	2.19
FZD pure tablets	Nil.	98.03 ± 3.65 mg tab^{-1}	96.38 ± 0.39 mg tab^{-1}	-	1.42
FZD suspension*	SPE[a]	41.62 ± 2.91 mg L^{-1}	44.12 ± 0.40 mg L^{-1}	-	2.29
FZD suspension**	SPE[a]	44.58 ± 1.27 mg L^{-1}	45.84 ± 0.29 mg L^{-1}	-	1.75
FZD blend tablets	SPE[a]	55.92 ± 1.70 mg tab^{-1}	57.85 ± 0.68 mg tab^{-1}	-	2.17
Feed A	SPE[b]	3.50 ± 0.03 mg kg^{-1}	-	3.71 ± 0.01 mg kg^{-1}	1.84
Feed B	SPE[b]	3.01 ± 0.03 mg kg^{-1}	-	2.87 ± 0.01 mg kg^{-1}	1.24
Feed C	SPE[b]	3.13 ± 0.03 mg kg^{-1}	-	2.88 ± 0.01 mg kg^{-1}	2.10
Feed D	SPE[b]	0.32 ± 0.03 mg kg^{-1}	-	0.47 ± 0.01 mg kg^{-1}	1.21
Feed E	SPE[b]	0.47 ± 0.03 mg kg^{-1}	-	0.31 ± 0.01 mg kg^{-1}	1.33

[a] Oasis® HLB cartridge

[b] Sep-Pack® NH_2 cartridge

[c] Standard deviation from three determinations for proposed method and reference method

[d] The official British Pharmacopoeia method [58]

[e] HPLC-DAD method [56]

[f] *t*-critical = 2.776 at 95% confidence [59]

*, ** FZD suspension from different brand

3.3.6 Recovery studies

The analytical recovery of the proposed method was evaluated by determining the recoveries of nitrofurans, after spiking two known amounts of nitrofurans in the sample. The recoveries of nitrofurans by this method showed satisfactory results which were in the range 96.94 - 105.80% indicating the proposed method was accurate (Table 3.27).

Table 3.27 Recoveries of nitrofurans in the presence of pharmaceutical and animal feeds samples

Sample	Extract procedure	Added ($mg\ L^{-1}$)	Found ($mg\ L^{-1}$)	Recovery (%) (mean ± S.D.)
NFZ cream	Nil.	2.00	1.94	96.94 ± 0.60
		4.00	3.89	97.18 ± 0.60
NFT tablets	Nil.	2.00	1.96	98.21 ± 0.60
		4.00	3.92	98.10 ± 0.60
FZD pure tablets	Nil.	2.00	1.97	98.66 ± 1.00
		4.00	3.86	96.39 ± 1.20
FZD suspension*	SPE[a]	2.00	2.03	101.27 ± 1.00
		4.00	4.03	103.13 ± 1.20

[a] Oasis® HLB cartridge

[b] Sep-Pack® NH_2 cartridge

*, ** FZD suspension from different brand

Table 3.27 Continued

Sample	Extract procedure	Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%) (mean ± S.D.)
FZD suspension **	SPE[a]	2.00	2.08	103.76 ± 1.00
		4.00	4.15	103.70 ± 0.60
FZD blend tablets	SPE[a]	2.00	2.05	102.46 ± 1.00
		4.00	3.86	96.46 ± 0.60
Feed A	SPE[b]	2.00	2.01	100.21 ± 0.60
		4.00	3.90	97.40 ± 0.60
Feed B	SPE[b]	2.00	1.94	96.94 ± 0.60
		4.00	3.82	95.48 ± 0.60
Feed C	SPE[b]	2.00	2.06	103.27 ± 1.00
		4.00	4.05	101.23 ± 1.20
Feed D	SPE[b]	2.00	2.06	102.90 ± 1.00
		4.00	4.23	105.80 ± 0.60
Feed E	SPE[b]	2.00	2.07	103.73 ± 1.00
		4.00	3.95	98.86 ± 0.60

[a] Oasis® HLB cartridge

[b] Sep-Pack® NH_2 cartridge

*, ** FZD suspension from different brand