CHAPTER 4

CONCLUSIONS

A flow injection impedimetric label-free immunosensor system has been developed for the detection of *Salmonella*. Antibody against *Salmoenella* was immobilized on the gold working electrode via self-assembled monolayer (SAM) of thioctic acid. When solution containing *Salmonella* was injected into the flow system, the binding between antigens and antibodies caused the imaginary part of impedance to increase and this was related to the amount of *Salmonella* which can be monitored using Autolab PGSTAT30. Parameters in the flow injection impedimetric biosensor system were optimized and the optimum conditions are summarized in Table 46.

Table 46 Summary of the optimum values in the flow injection impedimetric biosensor system.

Parameters	Optimum
1. Regeneration solution	20 mM NaOH
2. Sample volume (μl)	400
3. Flow rate (μl/min)	50
4. buffer	100 mM Sodium phosphate buffer saline pH 7.4

These optimum conditions were employed for the detection of *Salmonella*. The impedimetric immunosensor provides good selectivity with short analysis time (≤ 25 minutes). The linear dynamics range is 2 to 24 cell/ml with a detection limit of 2 cell/ml. This developed system gives lower detection limit than some other reports (Table 47). Although a lower detection limit of 1-2 CFU/ml was accomplished by Ruan *et al.* (2002), the analysis time of their system is about 10 hours. This is because they detected viable *Salmonella typhimurium* in a selenite-

cystine medium by monitoring oxygen consumption with cyclic voltammetry. Therefore, the technique is sensitive because even 1 colony of bacteria in the sample can later increase in the medium. It is possible for this developed impedimetric immunosensor to obtain a lower detection limit (less than 2 cell/ml) if we increase the sample volume but this will prolong the analysis time.

Table 47 Detection limit reported for the determination of *Salmonella*

Technique	LOD (CFU/ml)	Linear range (cell/ml)	Reference
Impedimetric	2	2 – 24	This work
Piezoelectric	350	10^2 - 10^7	Pathirana *et al.*, 2000
Piezoelectric	~ 10^5	$5.3x10^5 – 1.2x10^9$	Ye *at al.*, 1997
Optical	~ 10^7	$1.25x10^5 – 2.5x10^6$	Mazumdar *et al.*, 2007

The matrix interference was also studied and statistically confirmed for various types of sample juices. The results showed that there was no interference in the sample ($P < 0.05$). However, the matrix gave a higher response than the response of blank of standard solution. Because of this the response of sample with no *Salmonella* (blank sample) was first recorded and used to subtract from the response of the sample to obtain the real response. Good percentage recoveries in the range of 79 $\pm$ 11 to 129 $\pm$ 11 were obtained which is better than those of other works which was found around 50% (Lucore *et al.*, 2000). From these results it is clear that this technique can be used to determine *Salmonella* in real samples. As an alternative the real response of the sample can also be found by performing standard addition.

Detection of *Salmonella* in real sample using this developed technique was done in 12 samples. *Salmonella* were found in 5 samples in the range of 6.3 $\pm$ 0.5 to 38.3 $\pm$ 0.8 CFU/ml. The samples were also analysed using AOAC standard method which confirmed the existence of *Salmonella* in the same 5 samples. One of the advantages of the immunosensor system over the standard method is that it can provide the amount of *Salmonella* while the standard method can only indicate whether there was any *Salmonella* in real sample. The developed biosensor technique

also use less analysis time, i.e. ≤ 25 minutes, while the AOAC standard method takes 4-5 days.

The preparation of the modified electrode is simple and can be reused up to 42 times during a 5 day period with good reproducibility (%RSD = 3.3). This helps to reduce analysis cost.

This developed technique can detect whole cell and cell fragments. This will be very useful in the case where cell fragment contains some toxin. This is another advantage over the AOAC standard method which can only detect the whole viable cells. Therefore, this developed technique is more effective than AOAC method due to its capability of detecting non viable cells.

For further research, the detection of viable *Salmonella* in food should be developed in order to be able to detect *Salmonella* which is harmful to human. This can be performed by detecting the amount of *Salmonella* based on the kinetic of bacterial growth. The amount of *Salmonella* at different time interval can be obtained and the increase is corresponded to the amount of viable cell of *Salmonella* contaminates in the sample.