Chapter 4
Performance Criteria

As for any analytical method, it is important to characterize a biosensor response. The performance criteria need to be evaluated depend on the purpose of the method (Taverniers *et al.*, 2004). Some of the important performance criteria are listed below.

4.1 Selectivity

This factor is the most important characteristic of biosensor systems. It is the ability to discriminate between different substrates and concerns the range of chemical species, which can interact with the sensor. Selectivity of a system depends on the nature of the biological sensing element (*i.e.*, enzyme, antibody, and nucleic acid) and its selectivity for the substrate, as well as on the operational parameters (Eggins, 1996; Mortari, 2004). For example an antibody can bind to only one compound (target analyte, antigen) or to target analyte and other compound that have properties similar to the target analyte (Eggins, 1996). The method for biosensor selectivity determination can be performed by measuring the biosensor response to interfering compounds, a calibration curve for each interfering compound is plotted and compared to the analyte calibration curve, under identical operating conditions (Thévenot *et al.*, 1999; 2001). The selectivity of the work presented in this thesis was tested by using different substrates that have physical or chemical characteristics similar to the target analytes.

4.2 Linear range, sensitivity and limit of detection

Affinity biosensor calibration is performed by plotting the response change *vs.* the analyte concentration or the response change *vs.* the logarithm of analyte concentration (Thévenot *et al.*, 1999; 2001). The linear range is the interval between the upper and lower levels of the analyte concentration that have been demonstrated to be determined with linearity (Figure 4.1) (Swartz and Krull, 1997).

Sensitivity is determined within the linear concentration range of the immunosensor calibration curve. The sensitivity is the slope of the immunosensor calicration curve, *i.e.*, response change/analyte concentration ((R/(C) or response change/logarithm of analyte concentration ((R/(log C) (Thévenot *et al.*, 1999; 2001).

The limit of detection (LOD) is defined as the lowest concentration of analyte in a sample that can be detected, though not necessarily quantitated (Swartz and Krull, 1997). There are several methods to evaluate LOD (Long and Winefordner, 1982; Miller and Miller, 1993; Taverniers *et al.*, 2004). Because of the characteristic of the responses, the LOD of the work presented in this thesis follows the IUPAC Recommendation 1994 (Buck and Lindner, 1994). It is defined as the concentration of the analyte at which the extrapolated linear portion of the calibration curve intersects the baseline-a horizontal line corresponding to zero change in response over several decades of concentration change (Figure 4.1).

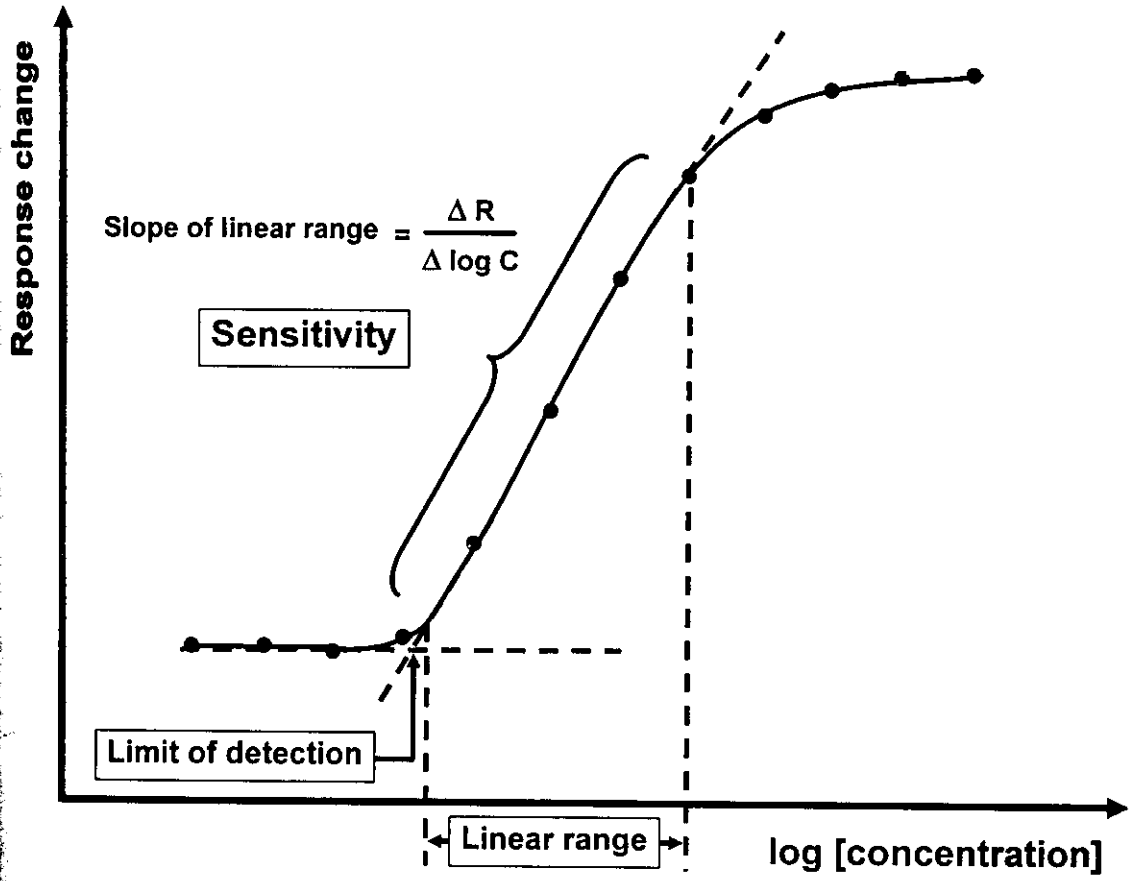

Figure 4.1 Schematic of a calibration curve showing relationships for determining linear range, sensitivity and limit of detection (Buck and Lindner, 1994; Eggins, 1996; Swartz and Krull, 1997; Thevenot *et al.*, 1999; Wang, 2000).

.3 Regeneration, stability and reproducibility

Another important issue for affinity biosensor is the question of ether the surface-immobilized probe can be regenerated without significant loss of tivity (Thévenot *et al.*, 1999; 2001). The interaction between target analyte and the mobilized biorecognition element is via non-covalently bound (*i.e.*, electrostatic

interactions, hydrogen bonding, hydrophobic interactions and Van der Waals interactions) (Byfield and Abuknesha, 1994; Gizeli and Lowe, 2002; Rabbany *et al.*, 1994). Thus, the dissociation of the target analyte-biorecognition element complex is possible by using regeneration solution. Regeneration allows surfaces to be reused many times, saving both time and money (van der Merwe, 2000). The regeneration of the biosensor system presented in this thesis was evaluated by considering the residual activity of the biorecognition electrode after regeneration. The criteria for regenerating the electrode surface is "if post-regeneration binding remains above 90 % compared to the binding efficiency before regeneration, the used conditions should be seen as adequate" (van der Merwe, 2000).

Although the activity of the affinity biosensor can be regenerated, all biological materials deteriorate in time, especially when they are removed from their natural environment. This means that one of the major drawbacks with biosensors is that the biological components usually has a fairly limited lifetime before it needs replacing. All developments of new biosensor include studies to show how the response of the biosensor to a standard sample changes with time (Eggins, 1996; Thévenot *et al.*, 1999; 2001). The operational stability of the biosensor system presented in this thesis was investigated by monitoring the change of the signal of biological element modified electrode at the same concentration of standard target analyte over a period of time.

Reproducibility is also another important factor with any analytical technique, but especially so with biosensors, where it is impossible to reproduce the quality of biological preparation as well as with ordinary chemical substances. Reproducibility is a measure of the scatter or the drift in a series of observations or results performed over a period of time (Thévenot *et al.*, 1999; 2001). In biosensor system the expected reproducibility between replicate determinations should be at least ± 5-10 % (the relative standard deviation, % RSD) (Eggins, 1996).