Summary and Conclusions

In the piezoelectric crystal based immunobiosensor applications, the most critical part of the biomolecular detection is to determine the resonant frequency in a precisely and reproducible manner. Frequency shift/change of only a few hundred hertz or less is expected from the formation of a receptor-ligand (antibody-antigen) complex on the crystal surface. Several factors affect the resonant frequency of the crystal, including the type of an oscillator circuit used and the surrounding environment. The basic understanding of the oscillator theory is thus an important aspect for this type of study. In the present work, some successful improvement in physical modification of sensor system and electronic circuitry and have been done in order to develop a stable and precise oscillator/frequency monitoring devices. By designing a single chip based frequency differentiating circuit, it became possible to obtained the absolute valuê of two frequencies (Reference and Sample) without any interferance. The oscillator circuit is capable of operating both in gaseous as well as in liquid phases, permitting on line operation of developed immunobiosensor system for the estimation of biomolecules. The use of standard AT-cut quartz crystal which has a temperature coefficient of about 1 ppm/^{o}C over a temperature range 10-50^{o}C, enabled better stability.

The study also demonstrate the usefullness of immobilization technique for uniform and stable binding of biomolecules on piezoelectric crystal

surface which lead to the development of a sensitive and reusable immunobiosensor. The developed immunobiosensor has a capability of quantifying biomolecules in the nanogram range both in liquid mode (on-line) and dry mode (off-line) without modification or labeling molecules with either costly reagents or radioactive isotopes. The absence of sample preparation is the most distinctive advantage of this immunoassay technique. The developed immunobiosensor could be easily automated with a flow injection system for on-line monitoring, which enabled repeated and continuous assays of biomolecules in serum. The device may also be upgraded to a multi-channel system, allowing simultaneous determination of different antigens in complex samples. The design and performance of the experiment is fairly simple, with minimum data acquition time compared to any analytical technique.

Immunoglobulin's (IgG, IgA, and IgM) and insulin molecules were selected in this study for their qualitative as well as quantitative estimation because of the importance of these biomolecules for the differentiation and diagnosis of various types of diseases. The system has been found economically more viable than any other established immunoassay techniques.

Although the experimentally obtained sensitivity of the immunobiosensor seems to be higher than that predicted by the Sauerbray equation, which was also reported by other authors (Beitness and Schroder, 1984; Lai et al., 1986), the utilization of previously generated calibration curves makes this fact relatively unimportant. The study demonstrated a linear standard curve for different insulin concentrations in the range of 10^{-1} mg/ml to 10^{-6} mg/ml. The line of best fit computed by least-squares regression method presented excellent correlation between insulin concentrations and the

resonant frequency shift ($r = 0.98$). A good reproducibility was observed as confirmed from the acceptable coefficient of variance (C.V. < 6%).

However, very few parameters applicable in this immunoassay technique such as antibody-antigen binding affinities, acoustic properties of adsorbents etc. are still unknown and need to be investigated thoroughly and implemented before such assays are used as routine immunoassay for biomolecular estimation. We have tried to optimise these parameters to a great extent to develop a real useful immunobiosensor for monitoring biomolecular concentrations.

In conclusion, the future use of the technique described in this work is very promising. However, the possible applications of piezoelectric crystal microgravimetric immunobiosensor in analytical or biomedical sciences seems only to be limited by the ingenuity of the analytical chemist.