

FLOW INJECTION ASSAY OF THE PATHOGENIC BACTERIA USING LECTIN-BASED QUARTZ CRYSTAL MICROBALANCE BIOSENSOR

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Aim of investigation

Development of a flow injection assay for the detection/identification of different strains of the pathogenic microorganisms *Helicobacter pylori*, *Campylobacter jejuni* and *Escherichia coli* using lectin-based QCM biosensor (Fig. 1).

Biosensing part

Immobilized lectins - Concanavalin A (ConA), lectins from *Maackia amurensis* (MAL), *Ulex europeus* (UEA), *Lens culinaris* (LCA), wheat germ agglutinin (WGA) - on the working surface of the transducer

Transducer

Gold coated quartz crystal (10 MHz) (Fig. 2)

Procedure of lectin immobilization

1. Thiolisation with 11-mercaptoundecanoic acid, off-line (24 h)
2. Activation with EDC/NHS (1:1), on-line (flow rate 15 $\mu\text{l}/\text{min}$)
3. Injection of lectin, on-line (flow rate 15 $\mu\text{l}/\text{min}$) (Fig.3)
4. Injection of ethanolamine-HCl for blocking, on-line (flow rate 15 $\mu\text{l}/\text{min}$)

Working principle of the biosensor

The biosensor makes it possible to identify the bacteria presence using the lectins immobilized on the surface of QCM crystal which bind specifically to the certain oligosaccharides present on the cell wall of the bacteria injected. Formation of the complex [lectin-sugar] on the surface of crystal leads to the decreasing of the oscillation frequency of the crystal due to the increasing of its mass. The following injection of regeneration solution makes it possible to remove the formed complex from the crystal surface to make it reusable and ready for the next experiment (Fig. 4).

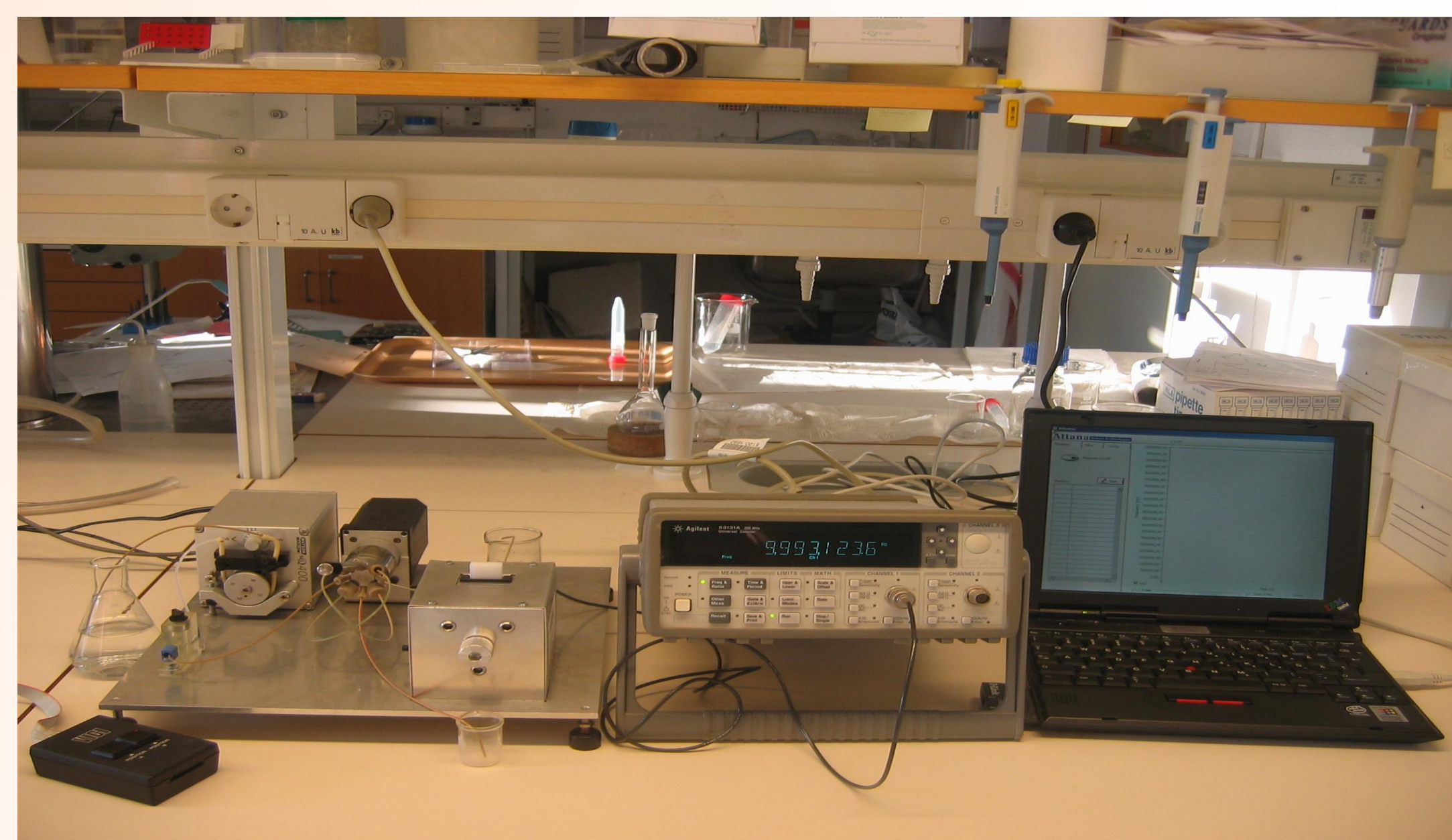


Figure 1. Flow- through QCM system (Attana 100, Sweden) integrated with PC



Figure 2. Gold coated quartz crystal electrode

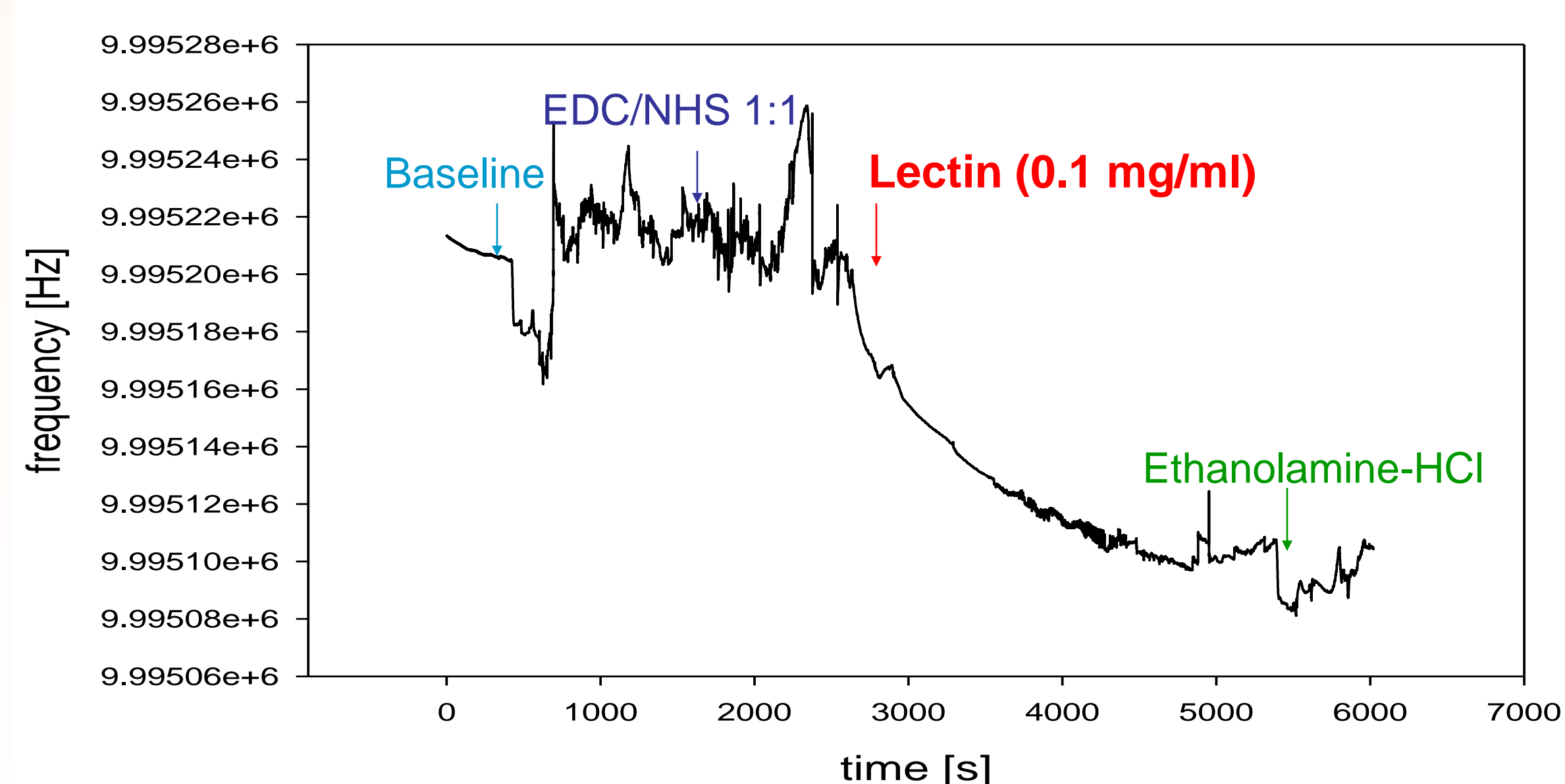


Figure 3. Immobilization of lectin (ConA) using amine coupling procedure

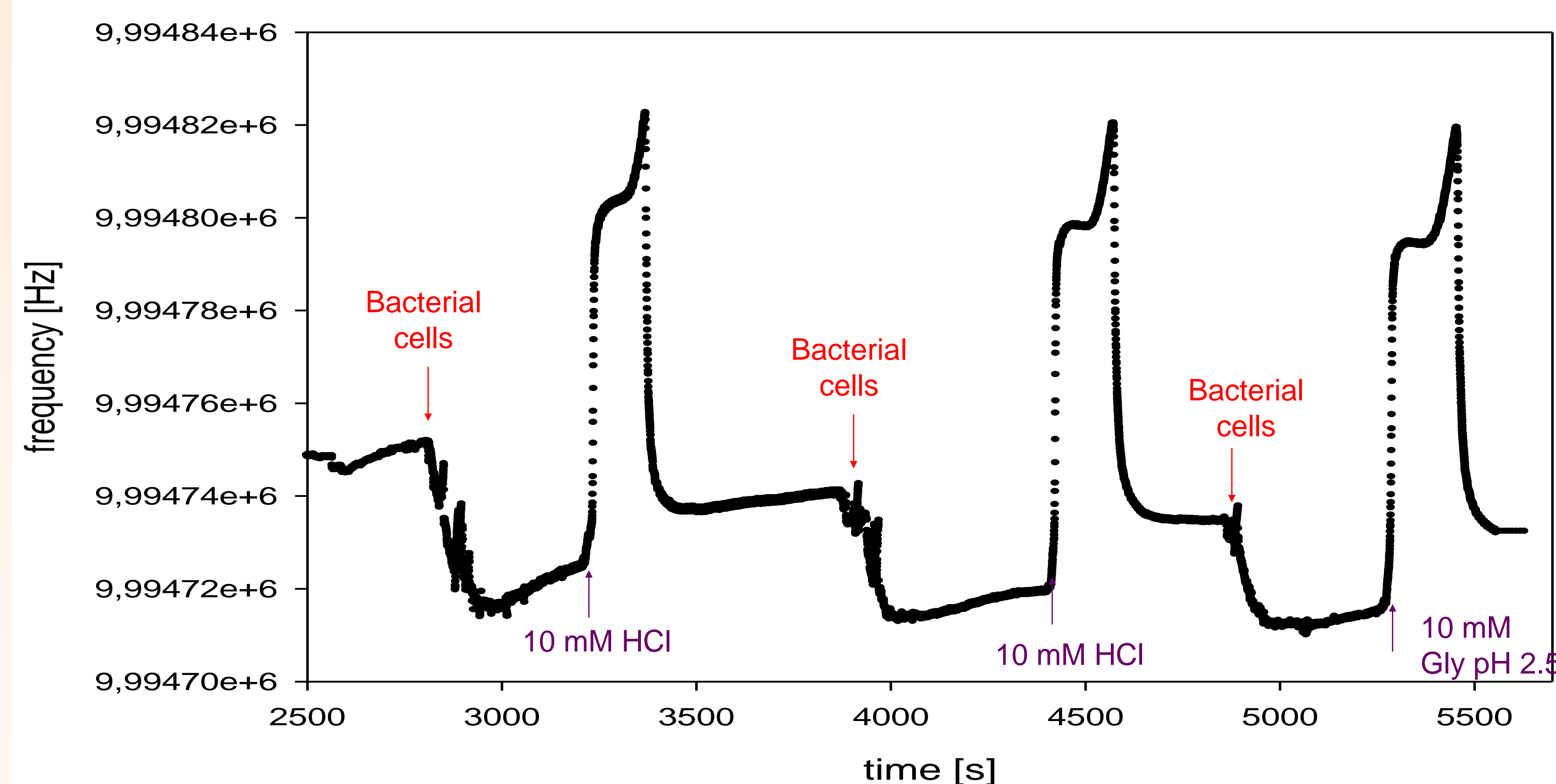
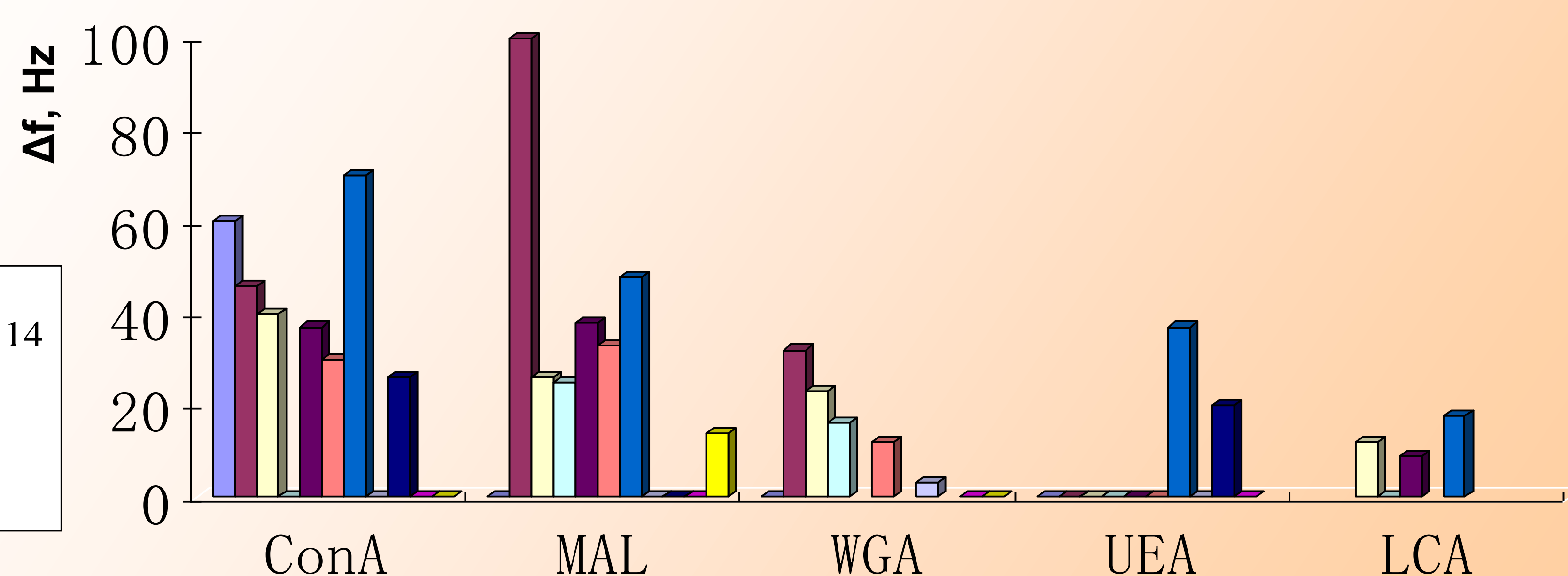


Figure 4. Response of flow injection lectin-based QCM biosensor. Injection of bacterial cells with following regeneration of the working surface of chip with 10 mM HCl, 10 mM Gly pH 2.5 (measurements are triplicated).

Flow rate is 40 $\mu\text{l}/\text{min}$;
dilution of the bacterial sample - 1:50;
injected volume - 50 μl .

Figure 5. The affinity interaction of the lectins immobilized on the surface of QCM chip with different bacteria

■ C.jejuni HS:3	■ C.jejuni HS:4
■ C.jejuni HS:6	■ C.jejuni HS:6 or NCTC 81114
■ C.jejuni HS:10	■ C.jejuni HS:41 or 370.95
■ C.jejuni O:19 or ATCC 43446	■ H.pylori 33
■ H.pylori 52	■ H.pylori 74
■ E. Coli DHa5	



Conclusion:

- A flow injection assay for the detection and identification of the pathogenic bacteria strains using lectin-based QCM biosensor has been proposed;
- The working conditions of the biosensor have been optimized;
- The affinity interaction of lectins immobilized with different kinds and strains of bacteria has been studied (Fig. 5).

Advantages of flow injection assay using QCM biosensor:

- Direct detection
- Label-free assay
- Sensitive
- Rapid (assay time ~ 40 min)