CHAPTER 7
CONCLUSION

Thanks to various innovative approaches, methods for broadening the comprehension of the biomolecular process are developing rapidly. The methods include the fabrication of sub micro-mechanical systems for the detection of biomolecules, an area that has been named nanotechnology-based biosensors.

As the concept of the nanotechnology-based biosensor is still in its inception, much additional work and endeavours are required to define and optimize the common configurations and processes that would allow excellent performances and add value to future applications. In the present study, we demonstrated a newer approach to the common task of the characterizing biomolecules and presented an analysis on derived practical applications that would permit users to calculate the constraints, limits, and potential advantages of such application.

In this thesis, we examined newer biosensors that have the potential of minimizing detection limits by means of understandable, miniature and strong devices. Among such biosensors, a QCM is an acoustical transducer that modifies mass changes on an oscillating quartz crystal resonator surface into an electronic or frequency signal; an IR spectroscopy is a physico-transducer that converts changes in transmittance peaks on the surface of GO-coated nanoporous PCTE membrane, and electricals signal describe the obstruction or aperture of a GO-laminates coated PCTE nanosieve where the analysed biomolecules have been blocked or hindered upon occurrence of biomolecular attachment. These biosensors devices can overcome the constraints of other systems based on mass-frequency, physico-IR peaks, and nanosieve-amperometric detections. In fact, while nanosieve detectors are severely constrained by the transient phenomenon triggering of signals, in the biosensor shown here the stable continuity of biomolecules in the nanosieve facilitates the detection of the transmembrane attachments.

The development of these devices was achieved through addressing different constraints to their productivity. The treatment and alteration of biosensor surfaces are the main conditions for the construction of good-quality and fully functional biosensors. The immunosensors enhanced with oriented immobilized antibodies were fabricated and checked on bacterial samples. The immobilization approaches proved to be important for the development of newer biosensors and their sensitivity and reusability. By finding appropriate conditions for immobilization of antibodies and conversion of their binding sites, the QCM biosensors were used for detecting target pathogens from samples. Mainly, we employed QCM biosensors for those applications presenting significant potential as label-free immunosensors. It should be observed that, in spite of the mastered tools, the interpretation of recorded signals is essential in case of piezoelectric biosensors and the theory should be further developed.

In this study, we exploited the supplementary of an anti-β-gal antibody to fabricate a rapid and accurate GO-coated QCM displacement assay for detecting of E. coli from samples. This research work presents a large sensitivity QCM-based biosensor using GO-
nano films. The sensitivity characteristics of GO-coated QCM-based biosensor were analyzed and quantitatively compared with other QCM biosensors, using the frequency investigation method. These results have already been published. We observed that GO-coated QCM biosensors have the highest frequency stability compared to other QCM in any biomolecules as described earlier in this thesis. The β-gal antigen showed a decrease in frequency of the order of 15.43 Hz, thereby illustrating a significant frequency change compared to conventional gold-coated crystals’ signals emitted upon antigen detection.

In summary, the QCM-based displacement assay we fabricated enabled detection of *E. coli* at concentrations as low as 5 ng.mL$^{-1}$ within 10 minutes, even in dirty environments that had the presence of structurally similar biomolecules. The described tool can also delineate the kinetics of biosensing, and based on this it is possible to develop a standard curve for on-site biosensing. Further, it can suggest a possible technique of the reported experimental event. Therefore, the present research has realized an effective method to construct high stability QCM-based biosensors using GO-coated nanomaterial such as biosensing nanofilms.

Further, in the subsequent articles, the recently published in-situ analyses are very accurate but they can be influenced by local small-scale processes, which are roughly simulated in the present models. On the other hand, remote biosensing analyses can complement the in-situ data well. Among the existing ground-based remote biosensing techniques, FTIR spectrometer has shown good prospects. The ground-based FTIR technique calculates total antibody-antigen quantities and constituents with high precision.

Regarding the IR spectroscopy-based biosensor, it is interesting to note that the GO-coated nano-film on the nanoporous PCTE membrane supports a new platform for the sensing of *E. coli*; this is perhaps the first instance of such application using an FTIR based nano-biosensor. The greatness of this method lies in the quick, label-free and strong detection of *E. coli* by a collection of the specific PCTE-GO-Ab-Ag attachment, as seen from distinct peaks on 3315 cm$^{-1}$, 1773–89 cm$^{-1}$, and 1600 cm$^{-1}$ as well as the characteristic fingerprints of the target (*E. coli*) pathogens. Hence, it may be said that our IR based biosensing tool enables a relatively rapid and specific detection through FTIR measurements. The fabricated nano-immunosensor based tool is selective, specific due to IR absorption, does not allow the linking of non-corresponding species of *E. coli*, and has a detection limit in the range of 100 μg.mL$^{-1}$ and 10 μg.mL$^{-1}$ and sensitivity up to 0.035 cd-gml$^{-1}$ cm$^{-1}$. The tool has potential to detect other analytes as well and is presently under development.

In the previous study, two components such as a biosensing platform and glass cell were fabricated individually and then put together, initially in the intermediate glass cell and then in the final implementation of the sensing device. One of the most challenging tasks was the biomolecular interaction in the upper side of the pore; while different antigens and antibodies interacted with large efficiency in the PBS solution; a reliable result was acquired only after the tuning of the aspect/ratio of pore/particles, the ionic concentration in PBS solutions, and the respective antibodies-antigens size. These tests of inter-biomolecular transmembrane chemistry made out of the sensing chamber were introduced to the assembled detector tool. During this process, the practicability of the nanosieve detector was recorded separately using a cylindrical electrophoresis glass cell, in which the solution was divided into two glass chambers by the simple PCTE and GO laminates coated PCTE membrane. Having a rather large (0.22-0.88 nm) GO laminates pore, as compared with other nanobiosensor approaches (Bhadra et al., 2016; Dikin et al., 2007; Nair et al.,
2012), led to very good SNRs. Indeed, the hindrance of the pore by antibodies-antigens interactions produced a mean 50% of the decrease in ionic current with values two orders of magnitude greater than the basic current.

This work mainly focused on the GO laminates coated PCTE membrane and its application for biomolecules detection using separation of ions through it. By various types of characterizations, both the structural and chemical merits of the prepared GO laminates were systematically observed. To utilize these peerless merits of GO laminates, a membrane coated with GO laminates was successfully developed for both biomolecules detection and ions separation. Fundamental detection processes were studied, including molecular sieving for H2 gas separations and underwater oleophobicity/low oil-adhesion for oil/water separation through GO membrane. Lastly, a facile process to tune the GO laminates chemistry and structure was explored, which showed the potential of GO-based nanomaterials for improved detection of biomolecules as well as separation performance.

First, we prepared ultrathin GO laminated membranes with thickness down to 41 nm on the PCTE membrane supports, which were the thinnest membrane realized in the previously published study. We found that, on account of the robust oxidative environment of GO synthesis, structural imperfections were present on GO the laminates. After conducting various biomolecules detection tests, the ultrathin GO laminates membrane developed high selectivity, while controlling a relatively high permeance of ions. This can be explained as the result of the selective structural imperfections in GO laminates that act as a pathway for ionic transport and block ions through the antibodies-antigens interactions over the laminates.

This application can be further extended to the separation of oil/water using GO laminates membranes. It was seen that flat GO membranes present amphiphilic and oleophobic properties in the air and underwater respectively. Compared with previous manuscripts reporting membranes for detection of biomolecules, the GO laminates membrane presented in this study demonstrated excellent performance in term of detection of biomolecules, filtrate flux density, and antifouling behavior. Lastly, we presented the feasibility of utilizing UV-Vis, to homogenously etch GO on the laminate and thereby appropriately tune the chemistry and morphology of GO layers in aqueous solution. Several characterizations, such as XRD, AFM, FTIR, and UV-Vis, confirmed that as a result of the UV oxidative test, both the chemistry and structure of GO layers were somewhat altered. This can be ascribed to the extra-induced hydrophilic functional groups and structural defects compared to graphite powder.

In summary, we fabricated a novel and flexible two-electrode electrochemical biosensor for gp140MS sensing, based on a PCTE membrane modified using GO-laminates with a receptor 2Dm2m protein. This research work demonstrated the selective ionic penetration merits of GO-laminates through a simple filtration process. The whole NPGO/membrane with homogeneous allocation was characterized by several techniques as described above. It may be said that this hybrid nanocomposite is a superior biosensing nanosieve platform with vital activator capability for the differentiation and sensitive detection of the gp140MS protein. Such differentiation and detection can be accomplished with other interfering non-supplementary protein (namely BSA) with 2Dm2m protein activity. Furthermore, making use of the 2Dm2m protein bind through EDC-NHS on the GOLaminates, we realized superior electrochemical outcomes for the gp140MS protein. Finally, our detection limit, sensitivity, and response time for gp140MS protein were 8.3 fM, 0.87
mA-mM$^{-1}$cm$^{-1}$ and 12 s respectively, all of which allow for the immediate sensing of HIV samples. Therefore this work provides a vital tool for medical kits or other applications. Also, the proposed nanobiosensor has great potential for the sensitive appraisal of other analytes supplementary to antibodies, in terms of its use of small biomolecules.