Chapter 6

Summary and conclusions

The results of the present study can be summarized under following categories: i) identification and characterization of the MAbs G7B5 and D2 binding components in *P. falciparum* parasite and infected cells, ii) development of an antibody-based biosensing platform for malaria diagnosis and iii) development of a hemozoin based malaria detection system using electrochemical and fluorescent based methods.

MAb G7B5, generated against *P. falciparum* parasite, inhibited *in vitro* parasite growth. Confocal microscopy revealed the reactivity of this antibody with the intracellular parasite no reactivity was observed on the infected cell membrane/surface. In immunoblotting, it reacted with a 78 kDa antigen of the parasite. To identify this antigen by sequencing of the immunoprecipitated component, the solubilization protocol was optimized. However, repeated attempts to immunoprecipitate this molecule was not successful. Another MAb, D2 raised earlier against the *P. berghei* infected red blood cells, was found to cross-react with the *P. falciparum* infected red blood cells (Pf-IRBCs), indicating the conserved nature of its epitope across the two species of Plasmodium. The D2 reactive component was found to be expressed on the surface of the Pf-IRBC at the trophozoite and the schizont stages of the parasite life cycle. Although, the antibody had shown mild cross-reactivity with the normal red blood cells (NRBCs) of *P.*

berghei, interestingly, in *P. falciparum* it clearly distinguished the infected cells (Pf-IRBCs) from uninfected cells (NRBCs).

For malaria diagnosis, to overcome the paucity of the biomarkers, *P. falciparum* infected cell based biosensor platform was developed. As summarized in Figure. 6.1, screen-printed electrode (SPE), modified by simple electrodeposition of the gold nanoparticle (GNPs) and MAb D2, was evaluated for its ability to detect the Pf-IRBCs. The binding of the cells to D2 on the SPE, was monitored using cyclic voltammetry and electrical impedance spectroscopy (EIS) based methods. The results showed EIS to be more sensitive for infected cells detection. The introduction of cells at varying cell concentrations produced well-resolved Nyquist plots leading to quantitative detection of Pf-IRBCs. The Nyquist plots were subjected to Randle's equivalent circuit modeling and the different electrical parameters were elucidated. A linear relationship between the charge transfer resistance (R_{ct}) and the logarithm of the concentration of the Pf-IRBCs was obtained over a concentration range of 10^2 cells/mL to 10^8 cells/mL. This part of the work provides a proof of concept for the impedance based electrochemical detection of infected red blood cells. The advantage of this strategy is the one-step preparation of GNP enhanced surface that can be used for the development of disposable sensors for rapid onsite detection of malaria.

In the third part of the study, the detection of hemozoin crystal inside Pf-IRBCs, using electrochemical and fluorescence based methods, has been described. The electrochemical study is focussed on finding an appropriate mediator to make the crosstalk between the hematin present in IRBC and the electrodes modified with rGO to enable sensitive early detection at low loads. The SPE modified with reduced graphene oxide (r-GO), in the presence of Tris(2,2′-bipyridine)dichloro ruthenium(II) hexahydrate as a redox mediator, was used to successfully detect the presence of hematin. The peak current obtained, using square wave voltammetry (SWV), showed a linear relationship with the hematin concentrations while achieving a sensitive detection of hematin up to 2×10^{-5} mM. The hematin extracted from the Pf-IRBCs also

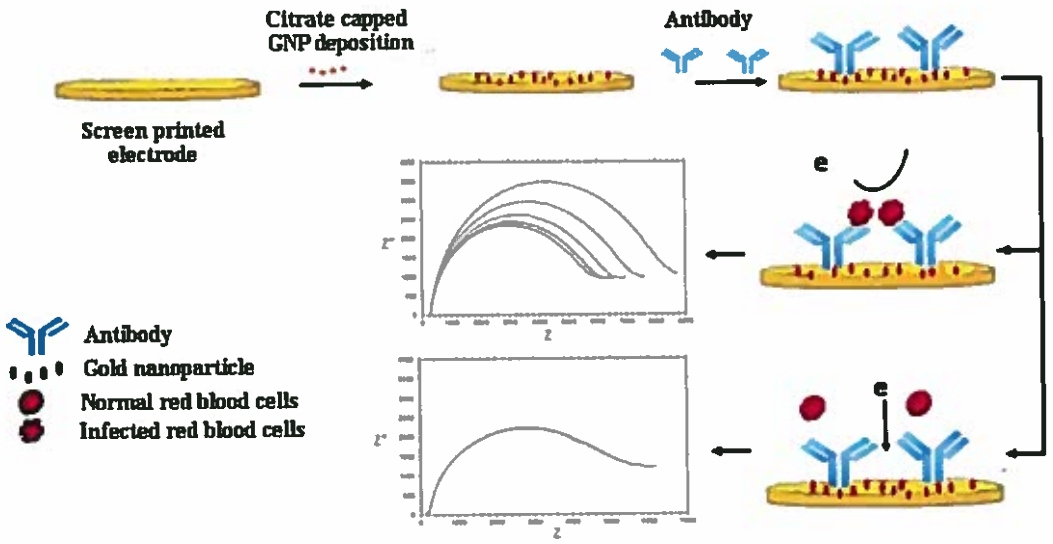

Figure 6.1: Schematic showing the detection of *P. falciparum* infected red blood cells on a GNP modified screen printed electrode using electrical impedance spectroscopy. If IRBCs are present the binding interaction leads to visible change in Nyquist plot. No electrical signal change is observed for NRBC.

exhibited a parasitemia dependent change in the SWV peak current. Since the hematin concentration correlates with the number of infected cells, the estimation of hematin concentration using this method can be used to determine the parasite load.

The protoporphyrin ring structure of Hemozoin has pi orbitals and is non fluorescent and has and absorption at 400nm. This particular properties of Hemozoin was used by us to device a fluorescence based quantitation of hematin. We employed PL (Photoluminescence) nanoclusters for the fluorescent based detection of hemozoin, as presented in Figure. 6.2. For this graphene quantum dots (GQDs) were used. The ability of hematin to quench the fluorescence of GQDs was evaluated where a dose-dependent quenching of GQD fluorescence was observed with hematin from both commercial and Pf-IRBC samples. Further, a linear relationship was obtained between the fluorescent intensity of the GQDs and the hematin concentration. Thus, electrochemical and the fluorescent based detection methods, using the r-GO modified SPE and GQDs, may provide a simple, cheap and sensitive platform for the detection of malaria infection. Added advantage of using this approach in malaria diagnosis is a requirement of a very

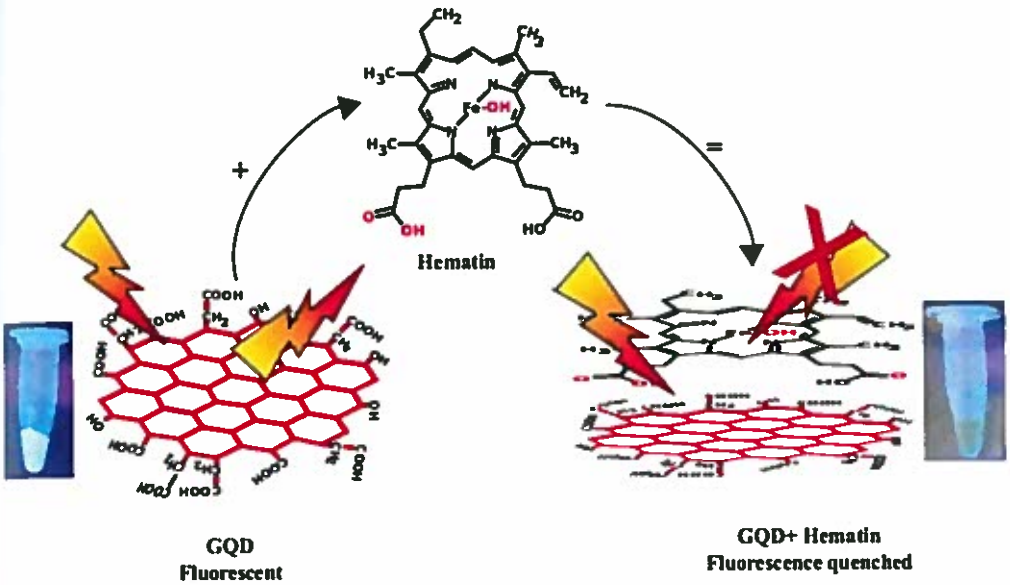

Figure 6.2: Fluorescence-based detection of hematin using graphene quantum dots (GQDs). The stacking interactions between the GQD and hematin lead to the fluorescence quenching of photoexcited states.

simple instrumentation.