
TABLE OF CONTENTS

	Pages
1. <u>INTRODUCTION AND OBJECTIV OF THE RESEARCH</u>	<u>1</u>
2. <u>THEORY</u>	<u>3</u>
2.1. BIOSENSOR	3
2.1.1. Mechanism of Biosensor	3
2.1.2. Analytical of piezoelectric crystal microbalance	5
2.1.2.1. Piezoelectric effect	5
2.1.2.2. The piezoelectric Crystal	8
2.1.2.3. Relationship between added mass and frequency shift	9
2.1.3. Biosensor in cell biology and pharmacy	10
2.2. MALARIA	12
2.2.1. Epidemiology	12
2.2.2. Biology of parasite	13
2.2.2.1. Invasion of merozoite	16
2.2.2.1.1. Structure of merozoite	16
2.2.2.1.2. Process of invasion	18
2.2.2.2. Release of merozoite	20
2.2.3. Chemotherapy and drug resistance	24
2.2.4. IMMOBILISATION OF ERYTHROCYTES FOR THE ANALYSIS WITH QCM	27
3. <u>METHODS</u>	<u>31</u>
3.1. PREPARATION GOLD SURFACE	31
3.1.1. Cleaning and adhesion of Quartz	31
3.1.2. Coating with Poly-L-Lysine	31
3.2. PREPARATION OF INFECTED ERYTHROCYTES	31
3.2.1. Malaria culture	31
3.2.2. Giemsa staining and parasitemia estimation	31
3.2.3. Dilution of malaria parasites	32
3.2.4. Synchronisation of parasites	32
3.2.5. Separation of schizont stages	33
3.2.6. Sample preparation for use with the biosensor	34
3.3. EXPERIMENT WITH QCM	35
3.3.1. Disinfection of the biosensor system	37
3.3.2. Sterility test for materials and reagents	38
3.3.3. Microscopic control of the quartz	39
3.4. CYTOMETRIC ANALYSE	40

TABLE OF CONTENTS (continued)

	Pages
4. <u>RESULTS AND DISCUSSIONS</u>	40
4.1. CHARACTERISATION AND OPTIMISATION OF THE POLY-L-LYSINE LAYER	40
4.2. OBSERVATION OF THE BIOSENSOR SIGNAL FOR 48 HOURS	51
4.2.1. Sterility	51
4.2.2. Stability of the PLL-erythrocyte layer over 48 hours	52
4.3. OBSERVATION OF INFECTED AND NON-INFECTED ERYTHROCYTES INSIDE THE BIOSENSOR SYSTEM	56
4.3.1. Concentration of the infected cells	56
4.3.2. Handling the erythrocytes infected with <i>P. falciparum</i>	59
4.3.3. Experiment controls	60
4.4. VALIDATION OF THE FREQUENCY MEASUREMENT FOR THE INFECTED ERYTHROCYTES AND THE NON-INFECTED ERYTHROCYTES INSIDE THE BIOSENSOR SYSTEM.	68
4.5. REINFECTION OF THE HEALTHY ERYTHROCYTES INSIDE THE EQUIPMENT	74
4.5.1. Re-infection control and optimisation	82
4.5.1.1. Efficiency of the re-infection method	82
4.5.1.2. Viability of merozoites	85
4.6. STUDY OF THE MEROZOITE INHIBITION	89
4.6.1. Test with Artesunat	89
4.6.2. Test with Chlorotonil	95
4.6.3. Test with proteases inhibitors	101
5. <u>SUMMARY</u>	115
6. <u>BIBLIOGRAPHY</u>	120
Appendix 1. Relationship Between Added Mass and Frequency Shift	135
Appendix 2. Properties of Some Important Merozoite Proteins in <i>P. falciparum</i>	136
Appendix 3. Clinical correlation of parasitemia	138
Appendix 3. Reagents and Equipments	139
