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Study and Presentation of Electromagnetic Method of Hemozoin Detection for Malaria Diagnosis

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Abstract

In this paper we examine the possibility of using the magnetic properties of hemozoin (especially its susceptibility) conceive a method for the detection of malaria. hemozoin presents its self in the form of a crystal and which constitutes a pigment synthesised by the malaria parasite with the aim of annihilating the toxic effect of hematine. In fact, after contamination the plasmodium dwells inside the red blood cells of the host so as to feed itself with the amino-acids obtained by degradation of hemoglobin. But during this "meal" the parasite liberates a waste product in the form of a complex made of porphyrin and iron. In order to prevent that noxious effect, the plasmodium polymerizes the complexes of porphyrin inside a digestive vacuole to finally produce a crystal of a hemozoin not toxic for him. This particle is previously paramagnetic because of the presence of iron in the red blood cells. Our study proposes a detection system of malaria, using the magnetic properties of hemozoin. The method consists in creating a variable magnetic field using coils considered as « primary» ; and applying this field in the blood which creates an induced voltage at the terminal of a coil of « measure». Consequently, the variation of this voltage is function of level of hemozoin in blood. In this approach, the module of detection is constituted of an electromagnetic circuit whose « primary» coils are supplied with alternative current and producing two antagonistic fields that have the same absolute value. The detection of hemozoin seems probable for a measured value of voltage, which may value some milli volts

Keywords: *Malaria; Hemozoin, Magnetism, Paramagnetic; Susceptibility.*

1. Introduction

Malaria is a most widespread infectious disease due to *Plasmodium Spp.* inoculated to men through puncture of the female anopheles. It has been proven that even not the indigenes do acquire immunity against malaria, so that without precocious diagnosis, infection still occurs, and may lead to dead [1].Hemozoin is a waste product of the malarial parasitic action on hemoglobin. Thus, its presence in the blood of an individual means the presence of the disease in that organism. The worldwide eradication trials of malaria have always failed because of the vector's resistance to insecticides and that of the parasite towards anti-malarial drugs. Actually, the reference technique, considered as "GOLD STANDART" for the diagnosis of malaria is microscopy through the objective 100X. [2] This diagnosis technique requires a specialized laboratory with qualified microscopists; and may cause hematomas due to blood collection. Additionally, the laboratory equipment (microscopes) required to perform microscopy is really expensive for the tropical dwellers, who constitutes the mostly affected population. Considering the limits of this technique, it is necessary to conceive novel malaria detection methods, which shall be as reliable as the actually approved methods; while extending their limits. It is in this sense that we made an attempt in exploiting the magnetic properties of Hemozoin in order to detect malaria parasites in the blood using an electromagnetic system. In the first section we present the proof that Hemozoin has an impact on the measured voltage. In the second section, we present the detection of the Hemozoin, magnetic particle; and finally, the results and the conclusion are presented in the last section

1.2 Diagnosis method of malaria

The reliable diagnosis of malaria, including the qualitative and quantitative detection of all the plasmodial stages in blood, is very important, especially in rural regions, where this disease responsible for the death of one to two million children per annum [3]. The comparison of different diagnosis approaches which include the conventional blood smear (CBS), photo-microscopy, the rapid diagnostic tests based on enzymes (RDT) and the PCR (Polymerase Chain Reaction) revealed a greater complexity of the malarial infection among the studied populations [4]. However, direct comparisons of these

methods illustrate differences in their sensitivity, specificity and their cost-effectiveness. Considering the RDTs which can be carried out quickly in remote areas, or the high sensitivity and specificity of PCR [5] used in well-equipped laboratory, these methods lose the capacity to evaluate the morphology of the parasite, which is very instructive about the parasite's stage. These comparisons prove that no approach can really satisfy all the diagnosis requirements of malaria.

1.3 Microscopy

This technique has been used for more than a century, and its sensitivity may be equal to 10 parasitized cells permicrolitre of blood. However it takes a lot of time to perform and it is also prone to a significant variability during its application, particularly with regard to the number of examined fields and the methodology used to determine the parasitemia [6].

1.4 Rapid diagnosis tests

These tests are based on the antibody-antigen reactions, followed by the revelation of the Ag-Ac complex through chromogenic. We have: Optimal-IT (PfLDH) and Parasight F (PfHRP II)

1.5 PCR (Polymerase chain reaction)

It is a molecular biology technique based on the selection and the amplification of a specific parasitic gene starting using specific primers of this gene. It has the advantage of being able to detect a precise strain of the parasite using specific primers of the gene or after digestion of the PCR product with specific restriction enzymes.

1.6 Methods of detection of the hemozoin by spectrometry (RMM)

There is also a detection device of Hemozoin which uses its magnetic property. This technique mainly consists in retaining β-hématin using a powerful and permanent magnet, this is because the Hemozoin has the same chemical properties as β-hématin.

1.7 Methods of detection of Hemozoin

During infection, the malaria parasites invade red blood cells and digest the proteinic part (globine) of the haemoglobin's molecule. The Heme, which is toxic to the parasite, is converted into insoluble Hémozoïn having crystals sticks shape. The transformation of diamagnetic oxyhemoglobin of weak-spin (Fe^{2+}) into paramagnetic Hemozoin of high-spin (Fe^{3+}) produces a change in the magnetic state and in the susceptibility, which was previously been used to concentrate the cells parasitized in order to improve detection [8]. Initially deposited in the erythrocytes vacuoles, Hemozoin is poured in suspension in the plasma following the later rupture of the parasitized erythrocytes' membrane, from where it is finally cleaned by leucocytes [9, 10].

2. The New proposed Approach

Our objective is to show that the current induced in a coil could be a function of the magnetic behavior of the blood medium. In other words, the induced potential could depend on the concentration of Hemozoin in the infected blood of a human being. Indeed, the magnetism of Hemozoin is low but detectable; there are magneto-optical methods which makes its measurement possible [22]. However we need a laser, a filter and especially that the solution to be analyzed be transparent [23]. The difficulties in detection are not related to the required sensitivity. Very simple methods make it possible to detect some nano-grams of magnetic substance. We obtain this precision by perfectly compensating the potential of an induction coil with that of another rolled up in the opposite direction [24]. A simple system makes it possible to obtain this effect. It is an induction bridge perfectly balanced, made up of three arms. Two arms located at the ends of the circuit will be intertwined by induction whorls (primary) and the central arm will be intertwined by whorls of coil (secondary or of measurement) at the boundaries of which we shall obtain the induced current. In addition, aiming at allowing passage of field lines through the blood medium, a notch is made on both sides of the central arm (*matter of balance*). Having this configuration, if the coils are provided with variable current (*so that they produce opposed fields to the equivalent absolute value, while remaining within the limit of saturation of the magnetic circuit)* and if the sample (blood) present in capillary tube goes through the circuit under the action of the peristaltic pump, then, any variation of the induced current is a function of the magnetic behavior of the blood medium. But a problem arises, that of the self-magnetism of the blood. Indeed, the magnetic behavior of the latter does not exclusively depend on its Hemozoin content. There are paramagnetic substances which are likely to mask Hemozoin self-magnetism. Moreover, diamagnetism will also produce a significant part of the magnetic background noise. However, taking into account its size, the Hemozoin is the only substance in blood which can be blocked by a gradient of magnetic field. If one makes that measurement within a capillary in which a continuous flow of the sample to analyze is circulating, it shall be possible to block, during a certain time, all the circulating Hemozoin in a section of the capillary by engaging a small electromagnet. All this accumulated magnetic substance will be pulled at new by the current of fluid, when the electromagnet is deactivated. When this concentrated zone in Hemozoin will attain the level of the detection module, a variation of potential will be observed at the boundaries of the measurement coil. Therefore, the detection system consists of two modules: the electromagnetic filter and the detector itself.

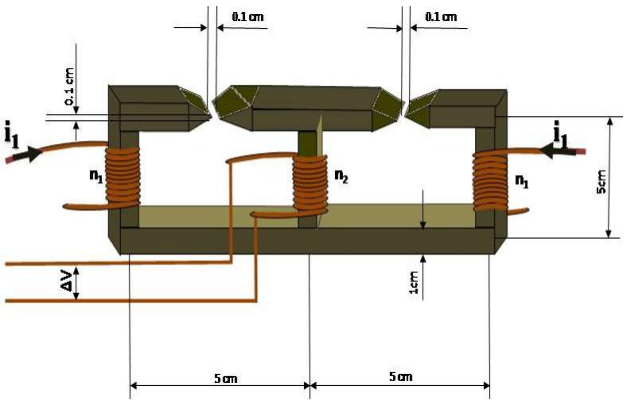

Figure1: Electromagnetic detector of Hemozoin

This paragraph is primarily devoted to the determination of the mathematical relation between the induced current and the concentration of Hemozoin in infected blood. Secondarily, this paragraph presents the implementation of the variations of the induced signal due to the magnetic behavior of the Hemozoin. The detection system is composed of two modules, which are the electromagnetic filter and the detector (figure1).

This module is a heterogeneous magnetic circuit and our study consists in determining the expression of the induced current (ΔV). To reach that point, we are use the equivalent diagram of electric circuit (*figure 2*) while taking into account of the analogy of the electric circuit/magnetic circuit.

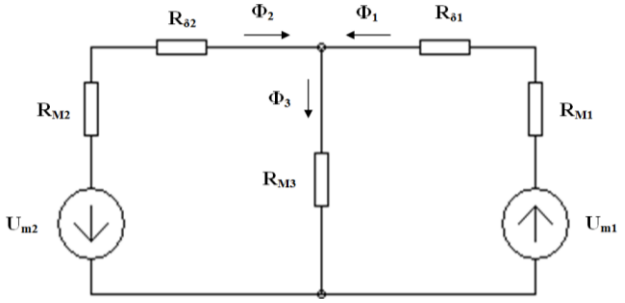

Figure 2: Equivalent diagram of the magnetic detector in an electric circuit

The value of the reluctance according to the nature of the material is given by:

$$R = \frac{\ell}{\mu s} = \frac{\ell}{\mu_0 \mu_r s} \qquad (1)$$

The circuit being heterogeneous comprises three types of materials: the soft iron core; the air (the air-gap) and blood:

In accordance with the geometry (section and length) and the nature of the core (μ_R = 500), we obtain the following values:

$$R_{M2} = R_{M1} = \frac{0.15}{4\pi \cdot 10^{-7} \cdot 500 \cdot 0.01 \cdot 0.01} = 2.39 \cdot 10^{6} H^{-1} \qquad (2)$$

$$R_{\delta 2} = \frac{0.001}{4\pi \cdot 10^{-7} \cdot (1) \cdot 0.01 \cdot 0.001} = 79.6 \cdot 10^{6} \cdot H^{-1} \qquad (3)$$

Generally, the air has the same magnetic properties as the vacuum (μ_r=1).

$$R_{\delta 2} = \frac{0.001}{4\pi \cdot 10^{-7} \cdot (1) \cdot 0.01 \cdot 0.001} = 79.6 \cdot 10^{6} \cdot H^{-1} \qquad (4)$$

The value of the reluctance of this portion relative to Hemozoin of the circuit depends on the susceptibility of Hemozoin.

$$R_{\delta 1} = \frac{0.001}{4\pi \cdot 10^{-7} \cdot (1+\chi) \cdot 0.01 \cdot 0.001} = \frac{79.6 \cdot 10^{6} H^{-1}}{1+\chi} \qquad (5)$$

By considering the electric diagram being equivalent (figure 2) and by applying the theorem of the mesh we obtain the following expressions:

:

$$U_{m1} = R_{M1} \cdot \phi_1 + R_{\delta 1} . \phi_1 + R_{M3} \cdot \phi_3 \quad \Rightarrow$$

$$U_{m1} = \left(R_{M1} + R_{\delta 1}\right) \cdot \phi_1 \cdot + R_{M3} \cdot \phi_3 \qquad (6)$$

$$U_{m2} = R_{M2} \cdot \phi_2 + R_{\delta 2} . \phi_2 + R_{M3} \cdot \phi_3 \quad \Rightarrow$$

$$U_{m2} = \left(R_{M2} + R_{\delta 2}\right) \cdot \phi_2 \cdot + R_{M3} \cdot \phi_3 \qquad (7)$$

The previously expressions permit to obtain magnetic flux expression in the central branch of circuit.

Combining the equation (6) and (7) we evaluate ϕ_3.

$$\left(R_{M1} + R_{\delta 1}\right) . \phi_1 + \left(R_{M1} + R_{\delta 1} . (1+\chi)\right) . \phi_2 + 2R_{M3}\phi_3 = 0 \qquad (8)$$

$$\Leftrightarrow R_{M1}\phi_1 + R_{\delta 1}\phi_1 + R_{M1}\phi_2 + R_{\delta 1}\phi_2 + R_{\delta 1}\chi\phi_2 + 2R_{M3}\phi_3 = 0$$

$$\Leftrightarrow R_{M1}\phi_1 + R_{M1}\phi_2 + R_{\delta 1}\phi_1 + R_{M\delta 1}\phi_2 + R_{\delta 1}\chi\phi_2 + 2R_{M3}\phi_3 = 0$$

$$\Leftrightarrow \left(\phi_1 + \phi_2\right) . R_{M1} + R_{\delta 1} . \left(\phi_1 + \phi_2\right) + R_{\delta 1}\chi\phi_2 + 2R_{M3}\phi_3 = 0$$

We know that

$$\phi_3 = \phi_1 + \phi_2 , \qquad (9)$$

Therefore, we have:

$$\left(R_{M1} + R_{\delta 1}\right) . \left(\phi_1 + \phi_2\right) - 2R_{M3}\phi_3 = -R_{\delta 1}\chi\phi_2 \qquad (10)$$

$$\Leftrightarrow \left(R_{M1} + R_{\delta 1}\right) . \phi_3 - 2R_{M3}\phi_3 = -R_{\delta 1}\chi\phi_2$$

$$\Leftrightarrow \left(R_{M1} + R_{\delta 1} - 2R_{M3}\right) . \phi_3 = -R_{\delta 1}\chi\phi_2$$

$$\Rightarrow \phi_3 = \frac{-R_{\delta 1}\chi\phi_2}{R_{M1} + R_{\delta 1} - 2R_{M3}} = \frac{-R_{\delta 1}\chi\phi_2}{R_{M1} + R_{\delta 1}} \qquad (11)$$

When χ <<< 1 we have :

$$R_{\delta 1} = R_{\delta 2} \text{ et } \phi_3 \approx 0$$

This equation shows well that in absence of Hemozoin in the blood, the flow through the axis of the central coil is almost null and consequently the induced current at the boundaries of the latter is null also.

We remain in the approximation where $\chi \prec\prec\prec 1$

The equations [7] et[11] make it possible to obtain Φ_3 according to the excitation current.

$$U_{m2} = \left(R_{M1} + R_{\delta 1}.(1+\chi) - R_{M3}.\frac{R_{\delta 1}.\chi}{R_{M1} + R_{\delta 1} - 2R_{M3}} \right).\phi_2 \qquad (12)$$

$$U_{m2} = \left(R_{M1} + R_{\delta 1} \right).\phi_2$$

$$\phi_2 = \frac{U_{m2}}{R_{M1} + R_{\delta 1}} = \frac{-U_{m1}}{R_{M1} + R_{\delta 1}} \qquad (13)$$

$$\phi_3 = \frac{-R_{\delta 1}\chi.}{R_{M1} + R_{\delta 1}}.\frac{-U_{m1}}{R_{M1} + R_{\delta 1}} = \frac{R_{\delta 1}\chi.n_1.i_1}{\left(R_{M1} + R_{\delta 1} \right)^2} \qquad (14)$$

The excitation current being sinusoidal, the induced current is obtained by applying the Lenz's law .The form of excitation current is given by:

$$i_1 = \hat{i}.\sin(\omega.t) \qquad (15)$$

This current has the shape of a sinusoidal signal of frequency 800 KHz and a maximum value of 0.1A (figure 3).

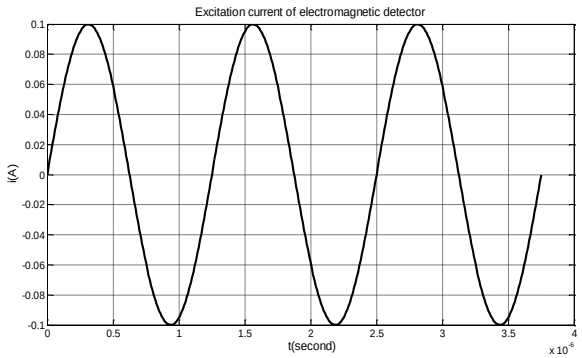

Figure3: Form and nature of excitation current.

The induced voltage is given by:

$$\Delta V = \frac{d}{dt}(\phi_3) = \frac{R_{\delta 1}.\chi.n_1.n_2}{\left(R_{M1} + R_{\delta 1} \right)^2}\frac{d}{dt}(i_1) = \frac{R_{\delta 1}.\chi.n_1.n_2}{\left(R_{M1} + R_{\delta 1} \right)^2}\hat{i}.\omega\cos(\omega.t) \qquad (16)$$

What enables us to deduce the expression the maximum value of the induced current

$$\Delta\hat{V} = \frac{R_{\delta 1}.\chi.n_1\hat{i}.\omega.n_2}{\left(R_{M1} + R_{\delta 1} \right)^2} \approx \chi.n_2.\omega.\frac{79.6.10^6.n_1.i}{\left(82.10^6 \right)^2} \qquad (17)$$

the excitation voltage is also obtained by applying the Lenz's law

$$U_{m1} = \left(R_{M1} + R_{\delta 1} \right).\phi_1 + R_{M3}\phi_3 \approx \left(R_{M1} + R_{\delta 1} \right).\phi_1 \qquad (18)$$

$$\phi 1 \approx \frac{U_{m1}}{R_{M1} + R_{\delta 1}} \qquad (19)$$

$$V_1 = \frac{n_1.i.\omega.n_1.}{R_{M1} + R_{\delta 1}} \approx n_1.\omega.\frac{n_1.i}{\left(82.10^6 \right)} \qquad (20)$$

Results

It is all about obtaining the sensitivity of the system to a presise frequency in order to define the supplying current, while maintaining constant the value of the current. Finally, it is all about presenting the evolution of the induced current according to the Hemozoin concentration. In order to have a sensitivity of 1mV for a Hemozoine concentration, we maintain the frequency excitement current's value of 800KHz

This curve presents the evolution of the entry current according to the frequency. Here it is to be noticed that this measured voltage is effectively a function of the frequencies and that the value of this signal is sensitive for a given frequency. For example to have a measured voltage almost equal to 1mV, it is necessary the frequency must be 800 KHz (figure 4).

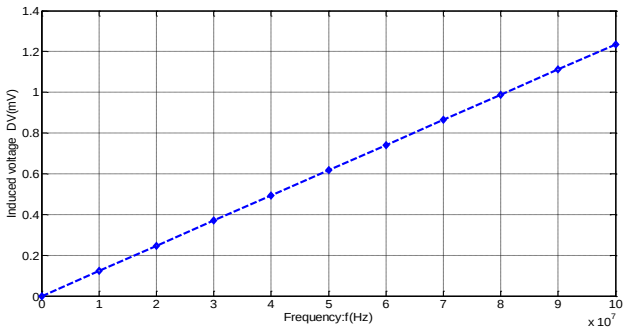

Figure 4: Evolution of input voltage according to frequencies for n_1=300 whorls; n2=3000 whorls

The preceding frequency (800Khz) makes it possible to obtain the necessary value of the excitation voltage (55Kv) to obtain a desired sensitivity.

The curve above presents the evolution of the excitation current according to the frequencies for N_1 = 300 whorls, N_2 = 3000 whorls; it shows how the entry current increases when the frequency is also increases. For example; this means that if the frequency is 800 KHz, the excitation signal of the circuit is practically 55KV (Figure 5).

With an excitation current of sinusoidal form having a maximum value of 0.1A, a frequency of 800KHz, we obtain an evolution of the induced current according to

the concentration (in millimol) of iron per liter, with N_1 = 300 whorls, N_2 = 3000 whorls (Figure 6).

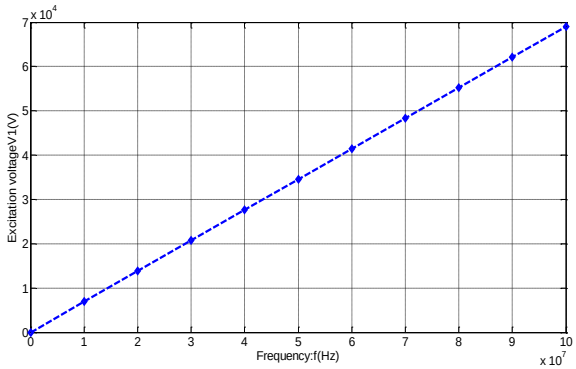

Figure 5: Evolution of excitation voltage in function of frequencies at n_1=300wires, and n_2=3000 wires

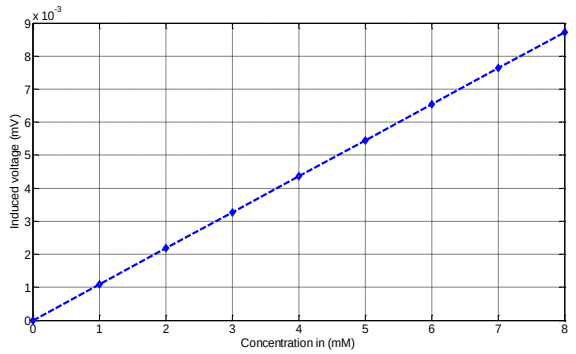

Figure 6: Evolution of induced voltage in function, of concentration (mM/) in hemozoine

The straight line (Figure 6) informs us about the proportionality ratio which exists between the induced current and the susceptibility of Hemozoin present within an infected blood.

Conclusion

This paper gave us the possibility to present a new electromagnetic method of Hemozoin detection in the blood; permitting the diagnosis of malaria. After having made a short outline of the prevalence of malaria in the world, we presented some methods of detection malaria and their respective limits. Concerning the method we proposed in our work, the development cycle of malaria informs us about the paramagnetic nature of Hemozoin. It is precisely this paramagnetism that proves to be the spinal cord of our study. In order to better understand the method of detection, we briefly presented the molecular magnetism and particularly, that of Hemozoin which led to its susceptibility. Then, we designed a magnetic circuit whose configuration allows obtaining the induced current dependently to the magnetic behavior on blood. The results obtained show that the induced current is proportional to the susceptibility of Hemozoin in an infected blood medium.

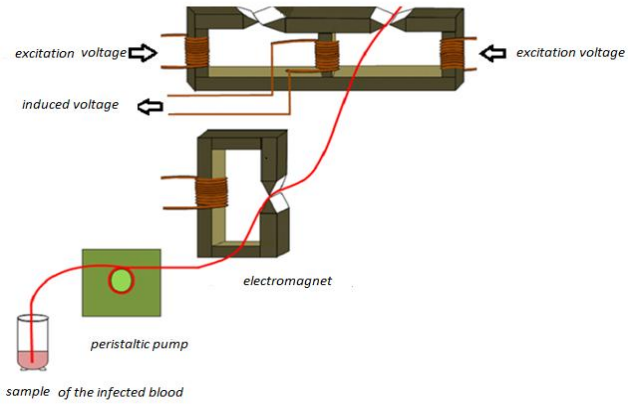

Figure 7: Electromagnetic system of Hemozoin detection

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