

# Magneto-Optics in the Service of Medicine - Diagnosis via the Cotton-Mouton Effect-

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**Abstract-**The Cotton-Mouton effect is presented and discussed as a means of detecting haemozoin and in the context of diagnosing malaria and schistosomiasis.

## I. INTRODUCTION

The Cotton-Mouton effect [1] is a magneto-optic effect arising as a consequence of the symmetry imposed when the constituent elements of a gaseous or liquid medium orientate in response to the action of the applied magnetic field. It manifests as a difference in the complex refractive index experienced by optical fields polarized parallel and orthogonal to the applied magnetic field propagating orthogonal to the field direction. Although recorded originally in gases and liquids in which the constituent molecules of the medium were the responsive elements, it may be applied more generally as an analytic technique to sense or probe the presence in fluids of any physical structures free and susceptible to the forces imposed by an applied magnetic field. In particular and, as will be shown, it has application in medical diagnosis.

Malaria and Schistosomiasis are the two most prevalent parasitic diseases of man and throughout much of the world they share a common habitat. Fifty percent of the world's population lives at risk of exposure to mosquito borne malarial infection [2,3]. Of the minimum 500 million cases occurring annually some 3 million are fatal with more than a third of these accounted for by infant (under 5yrs) mortalities in Africa alone. In recognition of these figures and the lack of appropriate diagnostics at a local level the WHO recommends the automatic treatment without diagnostic confirmation of all infants presenting febrile in areas where malaria is prevalent. Many of these treatments are of course unnecessary and the resulting over prescription is a major contributory factor to the development of drug resistance in plasmodium falciparum infection. Schistosomiasis similarly infects an estimated 200 million people but unlike malaria is commonly asymptomatic and less medically aggressive [4].

Although the parasites responsible for these infections are very different, both feed during the blood or erythrocytic

phase of their lifecycle within man on the oxygen carrying haemoglobin contained in red blood cells (RBC's). To digest the haemoglobin molecule and access the useful protein (globin) component both parasites have to render harmless the iron containing haem component which is otherwise toxic to them. This they achieve by converting it to insoluble crystals of haemozoin either retained in the malarial food vacuole or released into the plasma. Haemozoin crystals are paramagnetic and may respond to an applied magnetic field and hence generate a Cotton-Mouton effect the magnitude of which becomes a linear function of the haemozoin concentration. On this premise it is possible to quantify the haemozoin content of a finger prick blood sample (25 $\mu$ l) by using an applied magnetic field to align any haemozoin crystals present and then measure the resulting optical dichroism. This is easily achieved by probing the sample with optical radiation modulated rapidly between orthogonal states of high purity linear polarisation when the induced dichroism produces an amplitude modulation proportional to the haemozoin concentration. The validity of this procedure as a diagnostic technique for malaria has already been demonstrated [5]. In this present work we report briefly on initial studies exploring the potential of extending the technique to include the diagnosis of Schistosomiasis and the effect Schistosomiasis may have on the diagnosis of malaria. In particular we address ways of differentiating between diagnoses of these two diseases made via measurement of a common reporting mechanism.

## II. RESULTS AND DISCUSSION

Although chemically and crystallographically identical, the haemozoin produced by malarial and schistosomal parasites is morphologically very different [6]. Haemozoin produced by plasmodium falciparum or the other malarial parasites ( $H_{z_{mal}}$ ) is always found in the form of very regular rods some 800nm to 1000nm in length and 100nm to 200nm in cross section.  $\beta$ -haematin (BH), an artificially produced chemical analogue of  $H_{z_{mal}}$  has the same form but the crystals tend to be slightly longer. In comparison, the basic

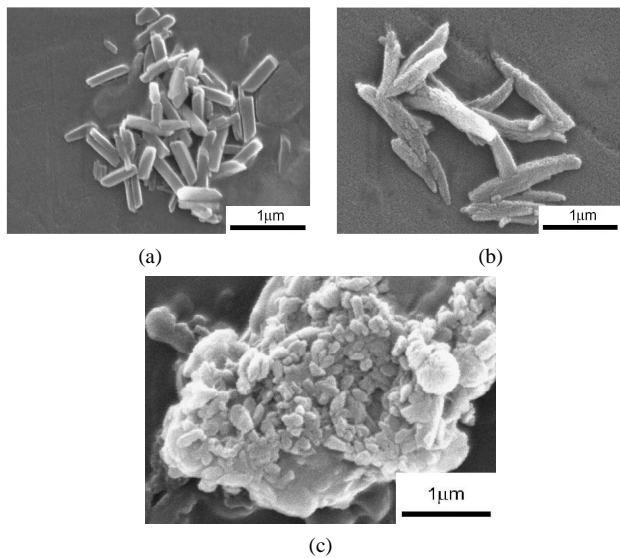


Fig. 1. Scanning electron micrographs of (a) haemozoin ( $H_{z_{mal}}$ ), (b)  $\beta$ -haematin (BH) and (c) schistosomal haemozoin ( $H_{z_{sch}}$ ) crystals, illustrating the different crystals' morphology.

structure of schistosomal haemozoin ( $H_{z_{sch}}$ ) is much smaller (50nm to 300nm) and ovoid or spheroid in form. It is however commonly found in the form of large (up to 2 $\mu$ m across) near spherical agglomerations of the smaller scale material. These different morphologies are illustrated in Figures 1 (a) through (c).

In suspension the magneto-optical response of crystals having these different morphologies to an applied magnetic field will reflect how the morphology influences both their magnetic and optical characteristics. Assuming that initially the crystals have random orientation in 3D-space because of the thermal energy of their environment then the optical properties of the suspension are isotropic. On application of a magnetic field the paramagnetic crystals become weak bar magnets experiencing a torque seeking to orientate them along the applied field direction. This is opposed by the

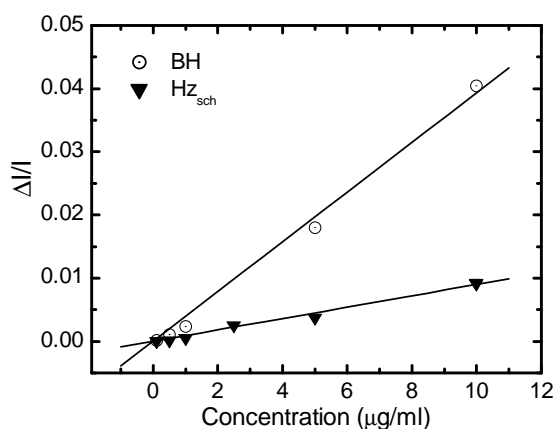


Fig. 2. The fractional change in transmittance  $\Delta I/I$  associated with magnetically induced dichroism on imposition of a 0.8 T magnetic field.

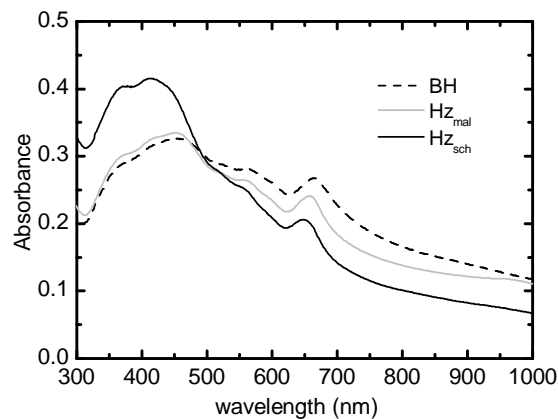


Fig. 3. Absorption spectra for 10 $\mu\text{g/ml}$  suspensions of  $\beta$ -haematin (BH), haemozoin ( $H_{z_{mal}}$ ) and schistosomal haemozoin ( $H_{z_{sch}}$ ) crystals in PBS pH 7.4.

thermal energy of their environment that constantly acts to randomize the assembly but as the crystals align with the applied field the medium develops a Cotton–Mouton effect. When all crystals are fully orientated with the field, any dispersion of haemozoin crystals behaves experimentally like a weak dichroic polarizer similar to Polaroid®. The magnitude of this effect, expressed as the fractional change in transmittance ( $\Delta I/I$ ) on imposition of a magnetic field, will be greatest for those crystals having the largest aspect ratio at equivalent crystal concentration – see Figure 2 and made more pronounced by the small (17% at the operating wavelength of 660nm) intrinsic difference in absorbance between  $H_{z_{mal}}$  and  $H_{z_{sch}}$  evident in Figure 3.

Moreover, since the orientating torque is also a function of the magnetic susceptibility and crystal length then the morphology differences between  $\beta$ -haematin,  $H_{z_{mal}}$  and  $H_{z_{sch}}$  will determine the form of the response curve as shown in Figure 4. This, in principle, offers a means of

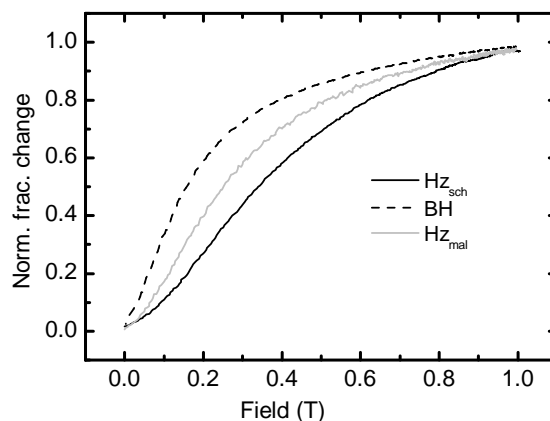


Fig. 4. The normalized fractional change in transmittance measured as a function of magnetic field for suspensions of  $\beta$ -haematin (BH), haemozoin ( $H_{z_{mal}}$ ) and schistosomal haemozoin ( $H_{z_{sch}}$ ) crystals in PBS pH 7.4.

differentiating between different haemozoin species within a common sample, with the fractional change for  $H_{z_{mal}}$  reaching towards saturation for magnetic field smaller than that required to saturate the response of  $H_{z_{sch}}$ .

Blood sample taken from patients living in regions where malaria and schistosomiasis prevail may contain either of the haemozoin species, or a mixture of both species depending whether patient may be infected simultaneously with the two diseases. The total haemozoin burden of the sample will consequently depend on the level of infection of both separate diseases and may also be dependant on the blood extraction site.

The schistosoma parasites are known to reside in the blood stream either in the perivesicular veins around the bladder or in the mesenteric veins around the liver [7] providing two fairly localized areas where schistosomal haemozoin is released into the blood stream. A large proportion of  $H_{z_{sch}}$  is also likely to be rapidly taken up by phagocytic white blood cells (WBCs), including fixed phagocytic cells in the liver, clearing it from the blood stream. In contrast malarial haemozoin is produced within the food vacuole of the malaria parasite, itself contained within infected RBCs. Intraerythrocytic  $H_{z_{mal}}$  is thus distributed throughout the blood circulation of the host with free  $H_{z_{mal}}$  crystals released into the blood stream as the malarial parasites complete each cycle of their multiplicative phase (every 48 hours) [8]. As with  $H_{z_{sch}}$  the free  $H_{z_{mal}}$  will be taken up by phagocytic cells throughout the vascular system and eventually cleared to the liver. With the finger being identified as an optimum site for malaria diagnosis with blood extraction in the form of a finger prick, extracted blood samples are therefore likely to contain a greater proportion of  $H_{z_{mal}}$ , in the form of free crystals and crystals contained within parasitized RBCs and WBCs, than schistosomal haemozoin ( $H_{z_{sch}}$ ). Furthermore, the smaller fractional change observed from  $H_{z_{sch}}$  compared to that measured from BH for the same concentration (figure 2) would indicate that in blood samples taken from the finger observed fractional changes would predominantly arise from  $H_{z_{mal}}$ , offering the possibility of malaria diagnosis in spite of possible schistosomiasis infection.

The validity of this technique, in particular its potential in differentiating between haemozoin arising from malaria and schistosomiasis infection remains uncertain as very little is known about the exact fate of haemozoin in the human body after acute disease other than its observation on necropsy in internal organs [9]. Further insight in the fate of haemozoin in relation to diseases' parasitemia will be gained from an upcoming field trial in Kenya later in 2008 intending to test this magneto-optical technique against current microscopy standards.

#### ACKNOWLEDGMENT

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