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Examining the Optical Properties of Monosodium Urate for the Detection of Gout Using a Magneto-Optical Device (MOD)

by

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Honors Project

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Abstract: Currently, there is a need for a cost-effective and more efficient option for gout diagnosis. Traditionally, gout is diagnosed by the presence of monosodium urate in blood or synovial fluid. The purpose of this research is to quantify the magneto-optical properties of monosodium urate crystals using a magneto-optical device (MOD). Characterization of these magnetic and optical properties is achieved through measuring the initial light intensity and the transmitted light intensity of a laser shone through a sample of monosodium urate with or without a static magnetic field. The comparison of the transmitted intensity through a solution of MSU crystals with a static magnetic field on or off allows for the analysis of the average extinction cross-section σ. Using our theoretical model under the simplifying assumption that absorption is dominant, we determined that $\sigma_x = 0.0127\ \text{cm}^3/\mu\text{g}$ and we determined σ_z using two different relationships which gave values of $7 \times 10^{-4}\ \text{cm}^3/\mu\text{g}$ and $0.0033\ \text{cm}^3/\mu\text{g}$. Due to the apparent difference in our calculations of σ_z, we determined that the extinction cross section for MSU crystals relies on both scattering and absorption interactions.

INTRODUCTION

In the United States alone as of 2019, around 9.2 million people are currently living with gout.[1] However, these numbers only reflect the people who have been diagnosed with gout. The most common symptoms of gout are joint pain, swelling, and redness which can be confused with other known conditions that affect joints, such as rheumatoid arthritis. On top of a wide range of nonspecific symptoms, gout remains difficult to diagnose in a primary care setting. Consequently, treating gout in a primary care setting often occurs after a patient begins experiencing acute symptoms like pain and joint swelling.[2] This difficulty in receiving a diagnosis is due to lack of access to gold standard diagnostic techniques in a primary care setting and the high cost of gout diagnosis with these techniques.

Currently, the gold standard for gout diagnosis is to analyze synovial fluid taken from the patient's affected joint with a crystalline polarizing light microscope.[3] Most primary care offices do not have crystalline polarizing light microscopy (CPLM) capabilities because these microscopes are expensive. As a result, the synovial fluid samples must be shipped to a laboratory, or the patient must visit a hospital that has trained professionals who can perform the needed CPLM to analyze the sample for the presence of MSU crystals. Other options for gout diagnosis include dual-energy computed tomography and ultrasound. However, these methods have the same disadvantages of CPLM in that they are costly, require trained professionals to run the equipment, and are largely unavailable in primary care settings.[4]

Due to this lack of easy access to currently available diagnostic methods, patients who wait longer to seek diagnosis after acute onset of gout symptoms will be less likely to be diagnosed and treated accordingly to prevent further flare-ups.[5] Without treatment, gout generally resolves in two weeks after the presentation of symptoms. However, getting a test directly after the onset of symptoms is optimal for an accurate diagnosis. It has been shown that the later synovial fluid is taken from a patient with a suspected gout flare up, the more likely it is that there will be smaller or even undetectable levels of MSU crystals in that fluid due to antibodies attacking them.[5] In these cases, crystalline microscopy as well as other methods may not be sensitive enough to determine the presence of crystals in the synovial fluid aspirate days after the acute onset of gout symptoms.

Fortunately, gout crystals have certain magnetic and optical properties that make this condition diagnosable using a table-top magneto-optical device (MOD). By using the MOD to measure the transmitted light shone through a solution of MSU crystals and to apply a static magnetic field to the sample, these specific magnetic and optical properties can be analyzed. Gout diagnosis with the MOD does not require a substantial amount of training and is a cost-effective option compared to other diagnostic methods. As a result of the lower price and less training required, the MOD is an accessible option for gout diagnosis in a primary care setting.

THEORY

When monosodium urate crystals form, they exhibit magnetic anisotropy.[6] Specifically, the components of the magnetic susceptibility tensor in the crystal basis are unequal. This inequality leads to the crystal having a hard axis (z-axis), which has a lower magnetic susceptibility and tends to align perpendicular to an applied magnetic field, and an easy plane (x-y plane), which has a higher magnetic susceptibility and tends to align parallel to an applied magnetic field. When the crystals are in an applied magnetic field, the c-axis, or long axis, of the crystal tends to align with the magnetic field at an angle of 69.3° as shown in Figure 1.[7]

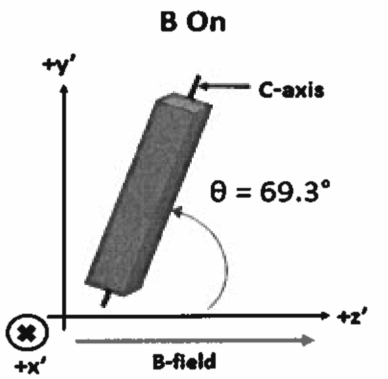

Figure 1 shows the orientation of an MSU crystal and its c-axis relative to an applied magnetic field.

Additionally, due to their geometry and crystalline structure, monosodium urate crystals exhibit birefringence and dichroism. Because MSU crystals are dichroic and birefringent, the absorption and scattering of light by MSU crystals depends on the relative orientation between the crystals and the polarization direction of the light incident upon them.[8] Specifically, dichroism and birefringence are characterized by a difference in the extinction cross sections for light polarized parallel to the hard axis (σ_z) and parallel to the easy plane ($\sigma_x = \sigma_y$).[8]

Experimentally, the MOD measures the incident light intensity, I_0, and the light intensity, I, transmitted through the fluid suspension of the crystals in the cuvette. By using the Beer-Lambert law,[9] the transmitted intensity, I, of a solution of dichroic and birefringent crystals is shown to be

$$I = I_0 e^{-\langle \sigma_\epsilon \rangle \ell c} \quad (1)$$

where $\langle\sigma_\epsilon\rangle$ is the average extinction cross section per crystal which is dependent on the crystal's orientation relative to the polarization direction, ϵ. In this equation, the length of the cuvette is defined as ℓ and c is the concentration of the MSU solution in terms of the number of MSU particles per volume. Importantly, Eq. 1 shows that the change seen in this light intensity, I, is directly caused by the change in the orientation of the MSU crystal due to the magnetic field, $\vec{B}$, being on or off. From the experimental data of these different measured light intensities, Eq. 1 can be used to determine $\langle\sigma_\epsilon\rangle$ from a semi-log plot of intensity versus concentration.

Our goal is to determine the extinction cross sections in the crystal basis, σ_x ad σ_z. To understand how this relates to our experimentally determined values of $\langle\sigma_\epsilon\rangle$ the laboratory, we must analyze the average crystal orientation and its effect on the extinction cross sections. In general, the extinction of incident light depends on both scattering and absorption interactions. However, from previous work on hemozoin crystals using the MOD, it was hypothesized that absorption cross-section is the dominant interaction for MSU crystals.[10] The same assumption was made here. Then, the extinction cross-section is approximately equal to the absorption cross-section. This absorption cross-section in the polarization direction is defined in Eq. 2. as

$$\sigma_\epsilon = k\, Im\{\alpha_{\epsilon\epsilon}\} \tag{2}$$

where k is the Boltzmann constant and $\vec{\alpha}$ is the polarizability tensor. Then, Euler angle analysis must be used with Eq. 2 to perform a basis transformation from the crystal reference frame to the laboratory reference frame using two rotations. After, the results of this basis transformation were analyzed for $\vec{B}$ on and $\vec{B}$ off. From this analysis, three equations for determining σ_x and σ_z for a single crystal from the experimental data were derived. These equations are:

$$\sigma_x = \sigma_x = \langle\sigma_{z'}\rangle_{B\ on}, \tag{3}$$

$$\sigma_z = 2\langle\sigma_{x'}\rangle_{B\,on} - \langle\sigma_{z'}\rangle_{B\,on}, \tag{4}$$

and

$$\sigma_z = 3\langle\sigma\rangle_{B\,off} - 2\langle\sigma_{z'}\rangle_{B\,on} \tag{5}$$

where $\sigma_{z'}$ and $\sigma_{x'}$ are the average absorption cross-sections in the laboratory frame.

METHODS

The analysis of MSU crystals was completed using the MOD pictured in Figure 2. The MOD was developed at Case Western Reserve University.[10] The MOD set up allowed for the use of vertically or horizontally polarized laser light to shine through the cuvette using a Thorlabs HL6358MG laser diode. To achieve the different polarizations of light, this monochromatic laser was sent through a rotatable linear polarizer. Then, this light was split using a 50/50 beam splitter, so that part of the light was measured by the first photodetector, and the other part of the light was sent through the sample. Then, intensity of the light sent through the sample was measured by the second photodetector. The

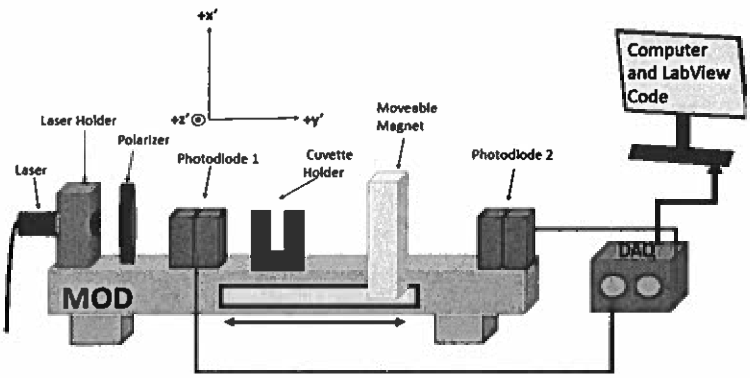

Figure 2 shows a schematic of the Kara Lab Prototype of the MOD used for research. On the MOD left to right, there is a laser, a holder for that laser, an adjustable polarizer, the first photodiode, the cuvette or sample holder, the moveable 7500 Gauss magnet, the second photodiode, and the DAQ and computer connections.

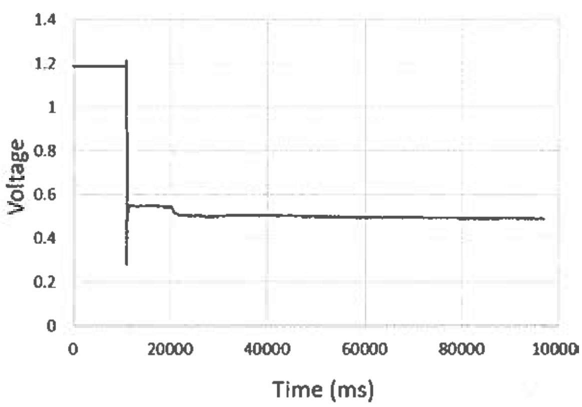

Figure 3 shows an analysis of horizontally polarized data where the first plateau indicates no sample is present, the second plateau is where the magnetic field is off and the sample is in the holder, and the drop in the voltage after the second plateau is where the magnetic field is on.

intensity of the light transmitted through the sample was analyzed over three distinct sections which can be seen in Figure 3. In Figure 3, the first part of the chart shows the light intensity before the sample was placed in the cuvette holder which was used for the initial light intensity in Eq. 1. The next section was the light intensity after the sample was placed into the MOD. The third section shows the change in light intensity after moving the magnets over to the sample due to the change in crystal orientation within the suspension to align with the magnetic field. The magnets on the MOD are fixed to a steel track that slides so that a static, external magnetic field of around 7500 Gauss in the horizontal direction can be applied to the sample in the cuvette holder.

The MSU crystals used in this experiment were acquired from InvivoGen as a dry powder and mixed in Dulbecco's phosphate-buffered saline (PBS) from EMD Millipore Company. Using the MSU crystals in solution, samples of MSU with concentrations ranging from 40 μg/mL – 180 μg/mL in steps of 20 μg/mL were created for data collection in the MOD. These crystal concentrations were determined to be large enough to be successfully detected by the MOD. Before each trial, the samples were vortexed using a Fisherbrand Mini Vortexer to ensure that the crystals were evenly mixed into the PBS. Before the sample was transferred from the sample holder to a standard cuvette which has a length, $\ell = 1$ cm, the cuvettes were first rinsed with an anti-adherence solution from Stem Cell Technologies to prevent sticking of the crystals to the cuvette. Then, the samples in the cuvette were inserted into the MOD and sat for approximately 10 seconds before the magnet was brought over them. A National Instruments Data Acquisition Tool (DAQ), model NI 9239 was used to collect the data for each trial from each photodetector. Then data was read into LabVIEW and evaluated in Excel.

RESULTS

Semi-log plots of intensity versus the concentration of the MSU samples are shown in Figure 4 (below). Figure 4a is a comparison of the transmitted light intensity versus

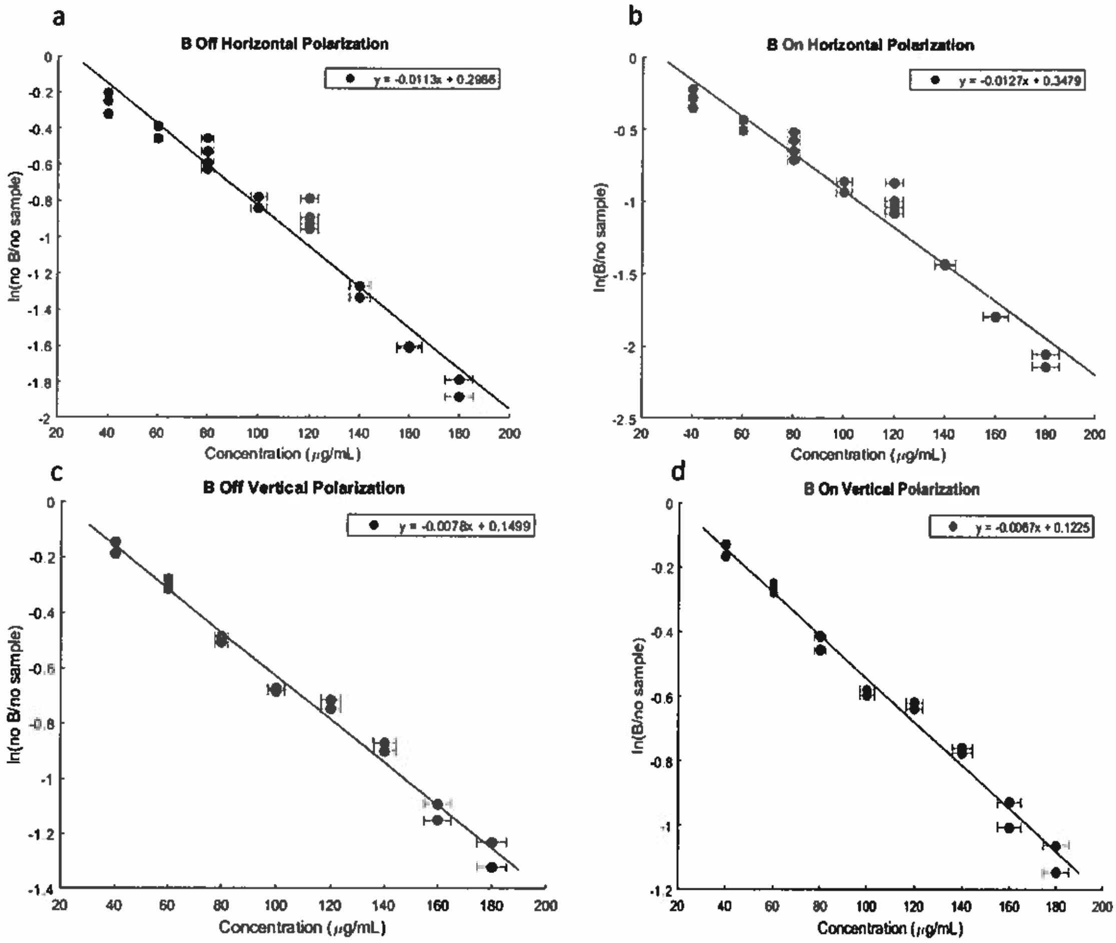

Figure 4: *a)* shows the graph of the natural log of the intensity with no magnetic field over no sample as a function of concentration for horizontally polarized light which follows a linear trend *b)* shows the graph of the natural log of the intensity with the magnetic field on over no sample as a function of concentration for horizontally polarized light which also follows a linear trend *c)* shows the linear slope of the natural log of the intensity with no magnetic field over no sample as a function of concentration for vertically polarized light *d)* shows the linear slope of the natural log of the intensity with the magnetic field on over no sample as a function of concentration for vertically polarized light

concentration for horizontally polarized light with the magnetic field off. Figure 4b shows the transmitted light intensity versus concentration for horizontally polarized light with the magnetic field on. Figure 4c and 4d show the comparison of the transmitted light intensity versus concentration for vertically polarized light with the magnetic field off and on respectively. Because these semi-log plots were found to be linear from experimental data, it was determined that the behavior of the absorption cross-section for MSU crystals is exponential, as expected from Eq 1. Linear fits of the semi-log plots in Figure 4 were used to determine the average values of σ in each case (see Table I). These slope values correspond to the exponential relationship between the transmitted light intensity and the initial light intensity as shown previously for solutions of dichroic and birefringent crystals like MSU. The average absorption cross-section can be calculated from these slopes. Three overdetermined equations: Equation 2, Equation 3, and Equation 4 from the basis transformation were used to determine σ_x and σ_z in the crystal frame from the experimental values taken in the laboratory frame.

Table I shows the calculated equations for the lines on the semi-log plots of transmitted light intensity versus concentration created from the experimental data.

Light Polarization Direction	Magnetic Field	Slope	R^2
Horizontal	Off	$-0.0113c + 0.2986$	0.953
Horizontal	On	$-0.0127c + 0.3479$	0.951
Vertical	Off	$-0.0078c + 0.1449$	0.986
Vertical	On	$-0.0067c + 0.1225$	0.984

Table II shows the calculated extinction cross sections in the crystal basis found experimentally using the average values of the average absorption cross sections of x' and z' from the laboratory frame.

Absorption Cross Section	Calculation from Average Values	Experimental Result ($cm^3/\mu g$)
σ_x	$\sigma_x = \langle\sigma_{z'}\rangle_{B\,on}$	0.0127
σ_z	$2\langle\sigma_{x'}\rangle_{B\,on} - \langle\sigma_{z'}\rangle_{B\,on}$	7.0000×10^{-4}
σ_z	$3\langle\sigma\rangle_{B\,off} - 2\langle\sigma_{z'}\rangle_{B\,on}$	0.0033

These values calculated from these plots are shown in Table II. Additionally, these values relate the average experimental absorption cross-sections in the laboratory frame to the absorption cross-section of one crystal in the crystal reference frame.

DISCUSSION

The values of σ_x and σ_z from the three overdetermined equations with two unknowns do not agree with each other. Specifically, the values of σ_z were found to have a percent difference of 78.8%. Because these overdetermined equations assume that absorption interactions dominate the extinction of light, we now believe that scattering is non-negligible in the extinction of light by MSU crystals. Other studies have hypothesized that scattering does play a role in the extinction interaction of MSU.[11] The main type of scattering mentioned in the study by Takeuchi *et. al.* is Mie scattering which is dominated by forward scattering of the light incident upon the MSU crystals. However, the group also found a large amount of backscattering of the incident light dependent the angle at which the light hit the MSU crystals. Further research and analysis of our data as compared to Mie scattering models is needed to improve our model for this research. Additionally, other common models of crystal scattering must be researched in order to provide a robust theoretical model to compare to current data from the MSU samples taken with the MOD.

In addition to this disagreement with theory, there could be systematic error in slopes due to the error in using the micropipettes used to measure out the concentrations of MSU crystals. Additionally, systematic error could be found in the average value of σ calculated from the slopes for vertical versus horizontal polarization with no applied magnetic field. Theoretically, these values should be the same because the MSU crystals are not in an applied magnetic field and are expected to be oriented randomly in both vertically and horizontally polarized light. Because of this randomness, the effects of dichroism and birefringence would cancel each other out and these values should be equal. However, in Table I, the slope for horizontally polarized light is almost twice that of vertically polarized light on the sample with no magnetic field in both cases.

Another uncertainty in the results could have been due to an observable increase in the temperature of the MSU crystal suspensions during mixing. As the samples were vortexed, the suspension heated up to the point where some of the PBS became condensed on the sides of the cuvette. This fog was visible to the naked eye and may have been left behind on the sides of the cuvette as the sample was transferred back to the sample holder. This loss of PBS from the suspension could have led to an increase in the expected concentration of MSU. Lastly, due to sample transfer from cuvette to sample holder and back, some of the PBS in the MSU crystal suspension could have been lost to evaporation. As a result, this evaporation could have also led to an increase in the concentration of the sample.

Lastly, MSU clumping and sticking to the cuvette was observed, leading to further uncertainty in our results. The cuvettes were rinsed between each trial, but if MSU stuck to the sides it may have been lost in the rinsing. This sticking was reduced using the anti-adherence solution. The anti-adherence solution was one step toward reducing the error in these trials for repeatability. However, these errors must be studied and further reduced in order to properly analyze the extinction cross-section of MSU crystals.

CONCLUSION

In conclusion, the average extinction cross-sections for horizontally and vertically incident polarized light for MSU samples in the presence of a magnetic field were found to be distinctly different. Because of these differences in the average extinction cross-sections, we are confident that the MOD can be used for MSU detection. However, due to the disagreement between our original model and experimental results, it must be concluded that proper analysis of extinction cross-section for MSU crystals relies on understanding both absorption and scattering interactions. As a result of this conclusion, the scattering of MSU crystals must be studied in detail. Then, a theoretical model and equation will be determined in order to compare theory including scattering to the data found experimentally in this project. After the theory and experimental data align, the extinction cross-section of MSU could be determined and used in the creation of a standard for detecting MSU with the MOD. Additionally, adjustments can be made to the mixing and measuring of samples to improve repeatability and reduce error in this experiment.

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This Honors Project has been approved and accepted by the John Carroll University Honors Program, in partial fulfillment of the requirements for an Honors Diploma.

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