PHYSICO-CHEMICAL CHARACTERIZATION OF SYNTHETIC HEMOZOIN CRYSTAL

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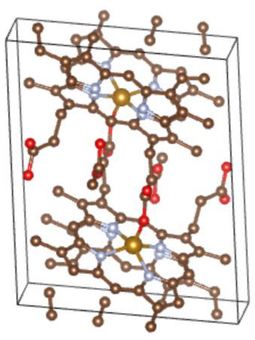
Introduction

Plasmodium is a parasite that is transmitted to man by the bite of a mosquito of the Anopheles genus that causes malaria. In one of the stages of the life cycle of the parasite in the human body, the plasmodium degrades hemoglobin and forms a crystal called hemozoin, as the main protection mechanism. For this reason, a complete understanding of the formation mechanisms and the physical-chemical characteristics exhibited by this crystal are very important to understand, to control and to detect the malaria disease [1-4]. Additionally, this crystal is also the target of several antimalarial drugs. An approach for this type of research is to manufacture in the laboratory, under diverse and controlled conditions and in the presence of various drugs, the synthetic analogue of hemozoin, which is called the β-hematin crystal. We present the kinetic studies, in aqueous medium, of conversion of hemin to β-hematin both in pure form and in the presence of chloroquine, a commonly used antimalarial drug.

Experimental procedure

Summary of one method for synthesis of β-haematin in aqueous medium



β-haematin crystalline structure [5,6]

10 ml of acetic acid+50 ml of distilled water → pH = 1.7 (or + 31 mg, 50 mg, 70 mg 100 mg CHQ)

↑ T → 70°C + 10 ml acetic acid (6h and agitation each 5 min)

160 mg of Hemin + 32 ml of NaOH solution were mixed (100-130 rpm) → 39°C (30 min)

795 mg of NaOH + 50 ml of distilled water

stirrer is turned off, stand 1h (β-haematin precipitates), supernatant pH = 3.3

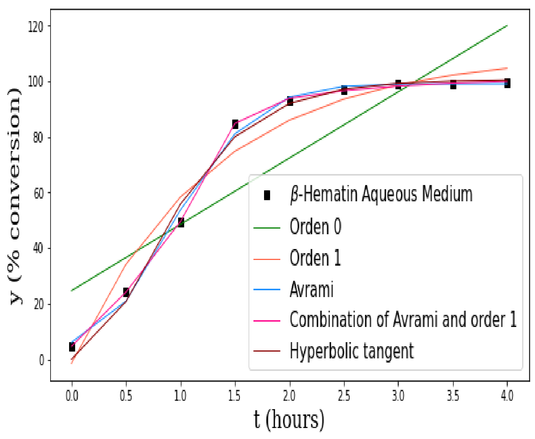
precipitated material: alternate washing: 3 times with distilled water, 3 times with ethanol

Drying: watch glass at 36 °C in oven for 48 h.

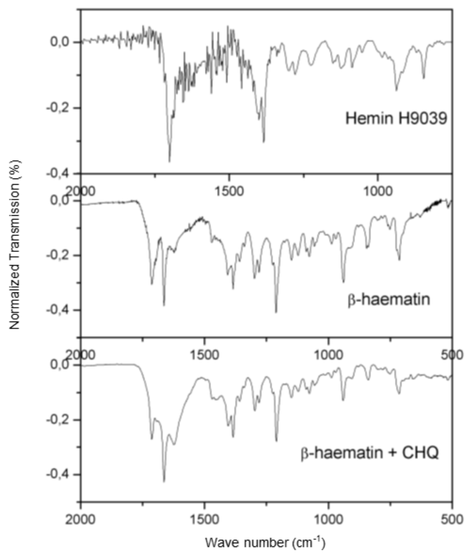
techniques: IR, Raman, XRD, Mössbauer, ppms.

Results

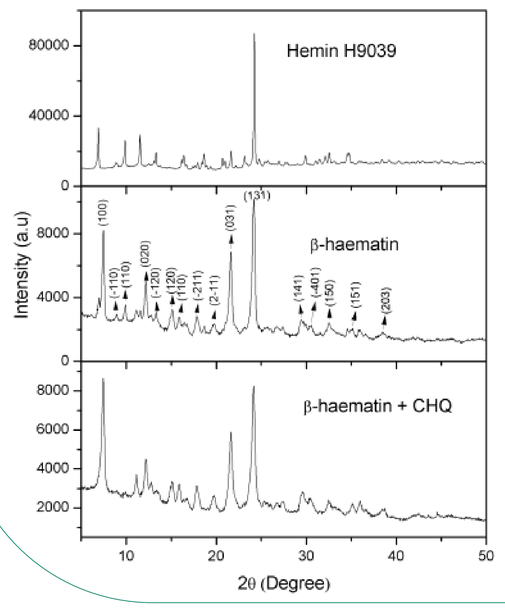
Kinetics of crystal formation



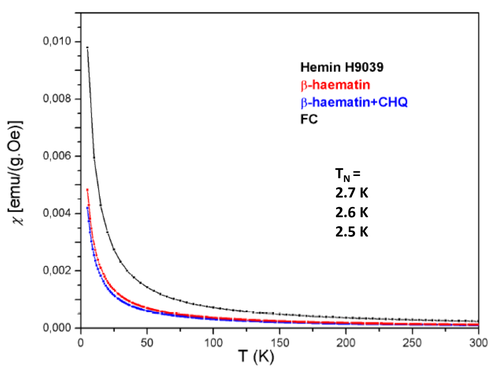
Infrared spectra



X-ray diffraction patterns

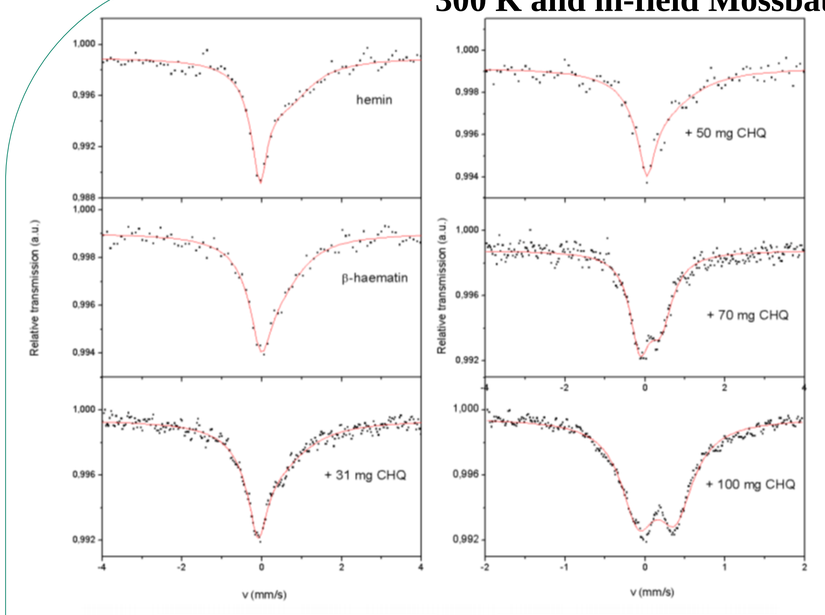


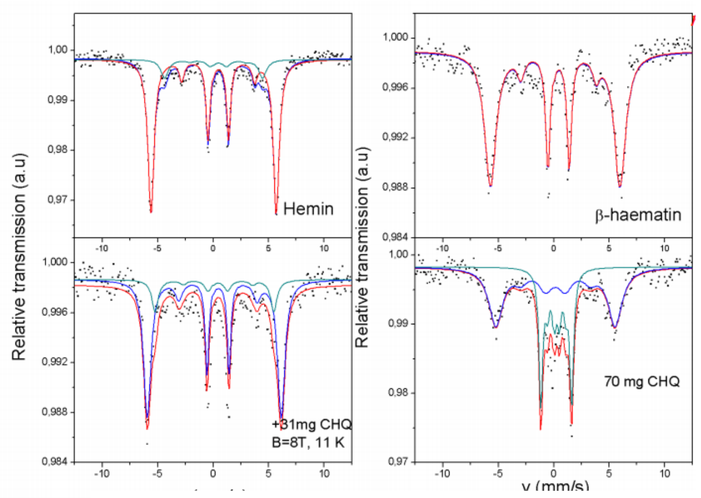
Specific susceptibility



The samples are antiferromagnetic with very low Neel temperatures (T_N < 2.6 K), indicating very weak magnetic interactions between the iron ions [7,8].

300 K and in-field Mössbauer spectra

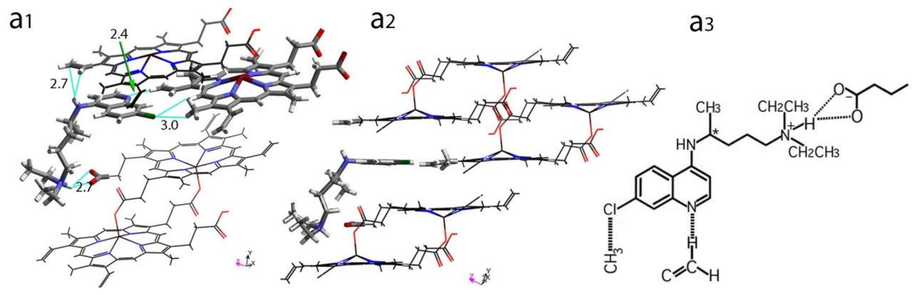




The change from asymmetric to symmetric (300 K and 77 K) spectra with increasing chloroquine concentration was associated with closer Fe3+ – Fe3+ distances.

In-field Mössbauer spectra for samples with the lowest chloroquine concentrations: the intensities of the intermediate lines were not equal to zero but had small values, perhaps suggesting weak magnetic interactions between the iron ions.

The average hyperfine field value almost equal in magnitude to the external applied field for sample with 70 mg chloroquine concentration pointed out to the presence of diamagnetic iron ions.



Molecular interaction between chloroquine and β-haematin. Figure taken from [1].

Conclusions

The results suggested that, under the conditions of the present experiments, the order from the best to the worst models for all kinetics of formation of β-haematin from hemin in aqueous medium are: lineal combination of Avrami and first order > Avrami > hyperbolic tangent > order 1 > order 0. XRD and FTIR showed the formation of β-haematin. The samples were all antiferromagnetic with low Néel temperatures. The 300 K Mössbauer spectra changed from asymmetric to symmetric doublets as the chloroquine concentration increased. This change was ascribed to closer iron-iron distances. The Mössbauer spectra in presence of external field for the β-haematins with the lowest chloroquine concentrations were adequately fitted with two sextets. The spectra for β-haematins with the higher chloroquine concentrations showed an additional sextet, with an average hyperfine field value almost equal in magnitude to the external applied field that may suggest the presence of diamagnetic iron ions. All these results support that this interaction may proceed via inhibition of crystal growing [1,2].

References

[1] S. Kapishnikov, et al. ChemMedChem 16 (2021) 1–19. [2] I. Weissbuch and L. Leiserowitz, Chem. Rev. 108 (2008) 4899-4914. [3] K. Olafson et al., PNAS 112 (2015) 4946-4951. [4] M.E. Ali and P.M. Oppeneer, Chem. Eur. J. 2015, 21, 8544 – 8553. [5] Pagola et al. Nature 404 (2000) 307. [6] K. Momma and F. Izumi, VESTA. 2019. [7] Fescenko et al. Physical Review Applied 11 (2019) 034029. [8] Y. Gossuin et al. Scientific Reports 7 (2017) 14557.

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