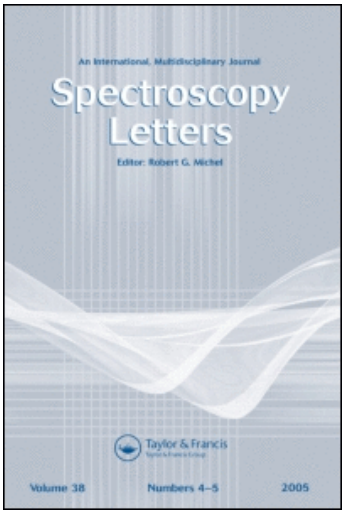


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Armando Paneque[a]; José Fernández-Bertrán[a]; Edilso Reguera[b]; H. Yee-Madeira[c]
[a] Center of Pharmaceutical Chemistry, Havana, Cuba [b] Institute of Materials and Reagents, Havana University, Havana, Cuba [c] School of Physics and Mathematics of IPN, Col. Lindavista, México, D.F., Mexico

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# Spectroscopic Characterization of Complexes Obtained by Mechanochemical Reactions of Hemin

**Armando Paneque,[1] José Fernández–Bertrán,[1] Edilso Reguera,[2,*] and H. Yee-Madeira[3,#]**

[1]Center of Pharmaceutical Chemistry, Havana, Cuba
[2]Institute of Materials and Reagents, Havana University, San Lazaro and L, Havana, Cuba
[3]School of Physics and Mathematics of IPN, Col. Lindavista, México, D.F., Mexico



## ABSTRACT

The solid state reactions of hemin with potassium acetate, KSCN, and $Ag_2SO_4$ were monitored using IR, Mössbauer and XRD techniques. These salts do not react at the peripheral propionic acid groups of hemin but form high spin complexes with hemin at the iron site. These

*Correspondence: Edilso Reguera, Institute of Materials and Reagents, Havana University, San Lazaro and L, Havana 10400, Cuba; E-mail: edilso@ff.oc.uh.cu; ereguera@yahoo.com.
#COFFA Fellow.



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complexes can be considered as ionic where the anions are coordinated to the metal through electrostatic interactions.

*Key Words:* Hemin; Hemin complexes; Mechanochemistry; Mössbauer; Infrared.

## INTRODUCTION

Hemin, protoporphyrin (IX) Fe(III) chloride, is the prosthetic group of important proteins such as hemoglobin, myoglobin, cytochromes and a variety of enzymes. It has been used as model to study the interactions of these proteins. Its complexes with basic ligands have been employed to treat porphyrias and anemia.[1,2] Hemin has a chloride ligand at the axial position of the central Fe(III) nucleus and two propionic acid groups in the periphery of the *pophyrin ring*. See Figure 1. Reactions at its Fe(III) site produce complexes, in which the nature of the Fe oxidation state and its spin configuration depend on the basicity of the ligands, their $\sigma$ and $\pi$ ability to donate or accept electrons, and the number of ligands, mono or bis, occupying the axial positions.[3,4]

The reaction of basic groups with the acidic peripheral groups is of importance in modifying the solubility and stability of the molecule in water or polar organic solvents. This is relevant to hemin use in pharmaceutical formulations.[1]

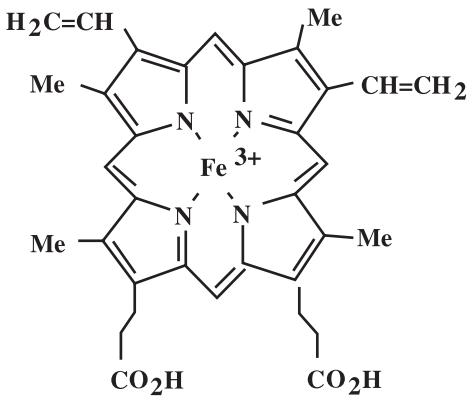


***Figure 1.*** Iron(III) protoporpphyrin IX moiety illustrating the presence of two peripheral $-CO_2H$ groups and the location of iron as complexing site at its axial positions, which are the reactions sites of hemin molecules.





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The reactions leading to the synthesis of these complexes have invariably taken place in solutions. The solvent media normally participates and in some cases interferes with the isolation of the products. We have found that the mechanochemical synthesis of hemin complexes can be a simple straightforward process, shortening the reaction time and simplifying the synthetic procedure.[5]

In this paper we present our results of milling hemin with slightly basic salts, such as $K^+$ $CH_3$-$CO_2^-$, KSCN and $Ag_2SO_4$, in an agate mortar and monitoring the solid reaction mixture by Infrared (FTIR), x-ray diffraction (XRD) and Mössbauer techniques, which allows the determination of the nature of the products and the mechanism of the reactions.

## EXPERIMENTAL

The samples of hemin, potassium acetate, KSCN and $Ag_2SO_4$ were analytical grade commercial reagents (from Sigma Chemical Co.). Their purity was tested by IR, XRD and Mössbauer techniques. The studied samples were prepared with 1:1, 1:3 and 1:4 molar ratios of hemin to ligands. They were milled in an agate mortar to a paste for 10 to 20 minutes and then were stored in a desiccator in the dark.

IR spectra were recorded in a FT-IR spectrophotometer (model Equinox 55 from Bruker), using the KBr pressed disk technique. The samples were also run as Nujol mulls to test that the grinding with KBr did not destroy the complexes.[6] XRD powder patterns were *recorded* in a Siemens D500 diffractometer using $CuK_\alpha$ radiation.

The Mössbauer spectra were obtained at 77 K with a constant acceleration spectrometer operated in the transmission mode and a $^{57}Co$ in Rh source. All spectra were fitted using a Blume-Tjon magnetic relaxation model[7] to obtain the values of isomer shift ($\delta$), quadrupole splitting ($\Delta$), linewidth ($\Gamma$) and relaxation time ($t_R$). The isomer shift values are reported relative to sodium nitroprusside at room temperature.

## RESULTS AND DISCUSSION

### Reactions of Basic Ligands with the Acidic Propionic Groups in the Hemin Periphery

Hemin is a molecule with two possible reaction sites, the peripheral $-CO_2H$ groups and the axial positions of the central iron atom, see Figure 1. The reactions of basic ligands with the propionic acid groups in the





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periphery of the porphyrin ring have a great importance on the physical properties of the hemin products. The binding of these acid groups with the iron(III) of the vicinal molecules as well as the hydrogen bonding interactions among themselves lead to strong association of the ferriprotoporphyrin IX molecules and to its low solubility in water and polar organic solvents.[8]

Figure 2 reports the IR spectra of the solid reaction mixtures in the 3000–1000 $cm^{-1}$ region where the interactions of the carboxylic groups with basic ligands can be easily detected by the disappearance of the strong sharp carbonyl band of the acid groups of hemin at 1706 $cm^{-1}$ and

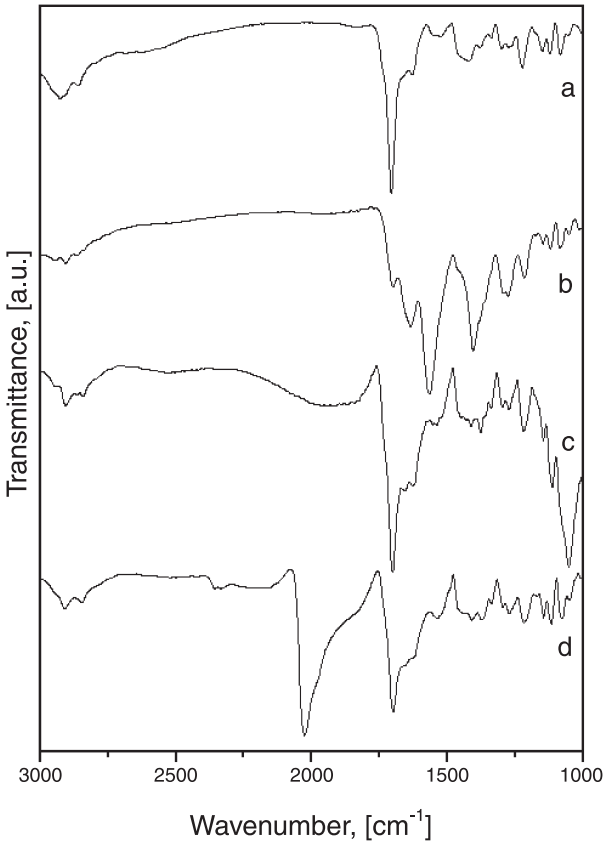


***Figure 2.*** IR spectra in the region (3000–1000) $cm^{-1}$ of: a) hemin; b) hemin+potassium acetate; c) hemin+$Ag_2SO_4$; and d) hemin+KSCN. All the mixtures were prepared in a 1:1 molar ratio of hemin to the salt.

its replacement by the carboxylate bands $\nu_{as}CO_2^-$ at 1555 $cm^{-1}$ and $\nu_sCO_2^-$ at 1400 $cm^{-1}$.[9] Examination of these spectra shows that these ligands do not transform the acid groups to salts, through a proton transfer reaction in solid state.[5] These salts have too low basicity to react with the acid groups of hemin since other salts such as KF, $NH_4F$, CsF, AgF, KCN and $Na_2S$ have been observed to react with these groups.[10]

## Coordination of Ligands to the Iron(III) Cation

The IR spectra of the milled mixture of hemin and potassium acetate shows two bands at 1660 $cm^{-1}$ ($\nu_{C=O}$) and 1211 $cm^{-1}$ ($\nu_{C-O}$) which are typical of the acetate anion coordinated to the iron atom in ferri-

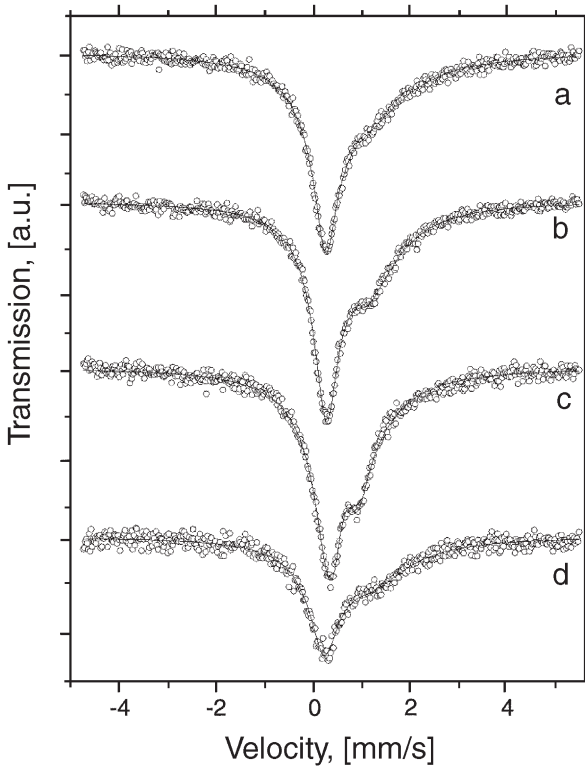


***Figure 3.*** Mössbauer spectra at 77 K of: a) hemin; b) hemin+potassium acetate; c) hemin+KSCN and d) hemin+$Ag_2SO_4$ (1:1). Indicated are the molar ratios of hemin to salts.

porphyrins (see Figure 2 a).[11] This IR spectrum is different from that of β-hematin which may be obtained from ferriprotoporphyrin IX-acetate solution.[12,13]

The IR spectra of the mixture hemin-KSCN show a band at 2042 $cm^{-1}$ ($\nu_{as\ (NCS)}$) which is characteristic of the anion $SCN^-$ coordinated to the iron atom in ferriporphyrins (see Figure 2 c).[14] When these reactions were carried out with molar ratios 1:3 or 1:4 (hemin:ligands) the IR spectra revealed excess of ligands, which indicates that only one ligand molecule interacts with the hemin iron atom.

The coordination of the ligands to the metal center was also monitored by Mössbauer spectroscopy and XRD. Mössbauer spectra of the milled mixtures are shown in Figure 3 and their Mössbauer parameters are reported in Table 1. The basic shape of these spectra is the same for the three derivatives, an unsymmetrical doublet with the higher energy peak broadened. Their isomer shift and quadrupole splitting values are typical of a high-spin (S = 5/2) $Fe^{3+}$ ion in an asymmetric crystal field.[15] The Mössbauer spectrum suggests that the thiocyanate anion probably is coordinated to ferriprotoporphyrin IX through the nitrogen atom; if it were coordinated through the sulfur, a higher isomer shift would be expected because the sulfur atom is larger and more polarizable than the nitrogen one.[16]

There was no Mössbauer spectral evidence for any other species present in the reaction products, in contrast to others reports.[17,18] The μ-oxo dimer gives symmetrical Mössbauer spectra and contains two high-spin iron(III) ions antiferromagnetically coupled through the oxygen bridge[17] while β-hematin gives a Mössbauer absorption consisting in an unresolved

***Table 1.*** Mössbauer parameters at 77 K of the solid state reactions of hemin with potassium acetate, potassium thiocyanate, and silver sulfate.

| Reagent or ligand* | δ** [mm/s] | Δ [mm/s] | Γ [mm/s] | $t_R$ [sec] | Assignment |
|---|---|---|---|---|---|
| $Cl^-$*** (Hemin) | 0.66 (1) | 0.82 (4) | 0.41 (1) | $2{,}28.10^{-9}$ | High spin $Fe^{3+}$ |
| $CH_3CO_2K$ (1:1) | 0.58 (4) | 0.60 (2) | 0.23 (3) | $2{,}15.10^{-9}$ | High spin $Fe^{3+}$ |
| $Ag_2SO_4$ (1:1) | 0.70 (3) | 0.96 (1) | 0.50 (2) | $1{,}35.10^{-9}$ | High spin $Fe^{3+}$ |
| KSCN (1:1) | 0.68 (3) | 0.85 (4) | 0.37 (3) | $1{,}88.10^{-9}$ | High spin $Fe^{3+}$ |

*: Indicated are the molar ratios of hemin to ligands;
**: Isomer shift values are reported relative to sodium nitroprusside;
() errors in **δ**, Δ and **Γ**.
***: Starting hemin (hemin chloride).





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quadrupole doublet above of 77 K[18] and a symmetrical resolved quadrupole doublet at lower temperature.[19] The β-hematin is a polymer formed by coordination of one of the propionate groups of Fe(III)-protoporphyin-IX to the Fe(III) center of the next and with hydrogen bonds between the resulting chains occurring via the second propionate group. Recently, the crystal structure determined using simulated annealing techniques have showed that the molecules are linked into dimers through reciprocal iron-carboxylate bonds to one of the propionic side chains of each porphyrin, and the dimers form chains linked by hydrogen bonds in the crystal. The β-hematin is chemically, spectroscopy and crystallographically identical to hemozoin or malaria pigment.[20]

The shape of the Mössbauer spectra of these three anions differs from that of anions which exert stronger ligand field, such as $CN^-$.[15] The weak perturbation of the metal electrons by these three ligands is due to the fact that they are weakly coordinated to the metal through predominantly electrostatic interactions.

The asymmetric broadening of the Mössbauer spectrum at 77 K can be explained on the basis of hyperfine interactions between the iron nucleus and its s electrons polarized by the $S_z = \pm 3/2$, $S_z = \pm 5/2$ d-shell electrons, together with a magnetic broadening due to the slowly relaxing $S_z = \pm 3/2$ and $S_z = \pm 5/2$ sub-states, which causes the observed absorption line shape (see $t_R$ values in Table 1).[7] A smaller $t_R$ value indicates a faster relaxation of the system. As expected, the smaller calculated $t_R$ value corresponds to the best resolved doublet (see Table 1 and Figure 3). An examination of the present Mössbauer results together with published data on pentacoordinated iron(III) protoporphyrin complexes[3] suggests the existence of certain dependence of the relaxation time on the bond properties of the involved ligands, however, as far as we know, no simple model is available to rationalize such dependence.

The interactions of the three salts with the iron(III) ion of hemin can easily be detected using XRD by the displacement of the chloride ion from the iron coordination sphere. Examination of the corresponding XRD patterns of the milled mixture shows the presence of KCl (see Figure 4) due to the displacement of $Cl^-$ for acetate and thiocyanate anions. The XRD pattern of the milled mixture of hemin-$Ag_2SO_4$ shows the presence of AgCl (see Figure 4) due to the displacement of $Cl^-$ for sulfate and $Ag_2SO_4$ in excess.

The formation of hemin-sulfate has been studied using ferriprotoporphyrin IX-methoxy and sulfuric acid but the isolation of the product is difficult.[3] The mechanochemical synthesis using hemin plus $Ag_2SO_4$ is efficient and allows a fast exchange of $Cl^-$ by $SO_4^{2-}$ anions, due to the high stability of AgCl.





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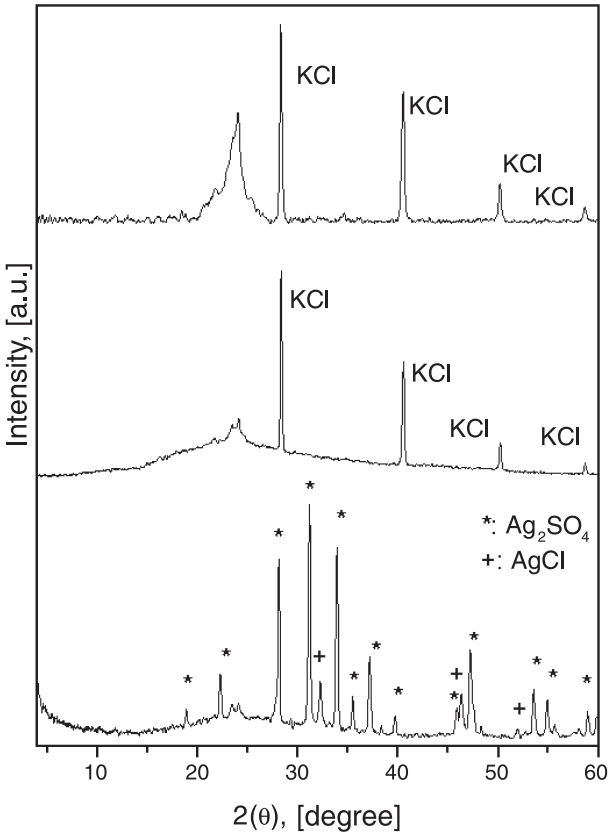


***Figure 4.*** XRD powder patterns of: a) hemin+potassium acetate; b) hemin+KSCN and c) hemin+$Ag_2SO_4$ (1:1). The displacement of $Cl^-$ from hemin is observed as formation of KCl or AgCl. In pattern c) unreacted $AgSO_4$ is also observed.

The XRD pattern of the milled mixture hemin-acetate does not reveal the presence of β-hematin[21] and corroborates the result obtained from Mössbauer spectroscopy.

The solid state reactions, as those discussed above, take advantage of the absence of solvent which always shows certain interactions with the substances to be reacted. Furthermore, the reaction in the solid state is relatively fast, requires a minimum of manipulation and it is practically free of side products. It is particularly useful to obtain hemin complexes through silver salt ligands at the iron position.

## CONCLUSIONS

The reaction of hemin with the studied basic salts in the solid state is an appropriate procedure to prepare ferriprotoporphyrin IX complexes. These salts do not react at the acidic carboxyl groups in the periphery of hemin but coordinate with the iron(III) ion with formation of high spin complexes that are easily detected by Mössbauer spectroscopy.

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