

## Point-by-point reply to referee # 1 for the manuscript:

*Insight into the structure of black coatings of ancient Egyptian mummies by advanced Electron magnetic resonance of vanadyl complexes*, by CE. Dutoit, L. Binet, H. Fujii, A. Lattuati-Derieux and D. Gourier.

We sincerely thank the reviewer for his detailed reading of the manuscript, which allows us to correct an error and to address some points that need to be clarified.

**Comment #1:** *Line 134: It is very rare for organic radicals to have g values less than 2.002. Here, the authors give 1.9994. What is the uncertainty in this number ? How was the magnetic field calibrated ? Is the line asymmetric ?*

**Response:** Thank you for this comment, which is absolutely right. We have accurately measured the g-factors on these samples as well as very recently on 14 other samples from a new corpus of mummies. The vast majority of values are around  $2.0037 \pm 2 \times 10^{-4}$ , with a few samples deviating slightly from these values,  $2.0032 \pm 2 \times 10^{-4}$  for the lowest, and  $2.0042 \pm 2 \times 10^{-4}$  for the highest. The magnetic field was calibrated with a sample of DPPH ( $g = 2.0037 \pm 2 \times 10^{-4}$ ). The organic radical line is mostly symmetrical both at X-band as well as at Q-band, indicating that the very slight asymmetry is not due to the anisotropy the g-factor (which is extremely small), but more probably to the influence of the neighbouring  $m_l = +1/2$  and  $-1/2$  hf lines of VO-P, which are very close to the  $C^0$  line and may slightly distort the latter. The g-value of radicals is now corrected in the manuscript (line 140, in red).

**Comment #2:** *It should be discussed why for the VO-nP species, not all hyperfine lines are visible. Presumably this is due to a distribution of hyperfine couplings resulting from ligation geometries that are less rigid than in porphyrins ?*

**Response:** In Figure 2, only the VO-nP lines that are most visible to the naked eye have been marked. Others can be seen by zooming in. There are two reasons why not all of them are visible: 1) several perpendicular transitions of VO-nP are hidden by VO-P lines; 2) the parallel lines are effectively broadened by a slight distribution of hyperfine couplings and g-factors (the broadening is more important at Q-band). However, simulations clearly have shown that VO-nP are vanadyls complexed by oxygenated ligands (Dutoit et al. 2020). The broadening of the parallel lines of VO-nP may indeed be due to a disorder effect linked to a much lower rigidity of the oxygenated ligands than that of the porphyrin rings. A sentence has been added (lines 148-149, in red) concerning the apparently missing lines of the VO-nP

**Comment #3:** *In the experimental section, include the RF power used in the CW-ENDOR experiments.*

**Response:** The rf power in the ENDOR coil is 10 W. This value has been reported in line 106 of the manuscript.

**Comment #4:** *Figure 4c uses on the horizontal axis the weight-percentage VO-P content of the samples. Describe how this was determined. Also, add horizontal error bar to Figure 4c.*

**Response:** Each sample introduced into the EPR tube was carefully weighed, and the intensities were expressed per unit mass,  $I_s/M_s$ , where  $I_s$  is the measured amplitude of a perpendicular hf line for the VO-P spectrum and  $M_s$  is the mass of the historical sample. Taking the Dead Sea asphalt, consisting of pure bitumen, as a reference, for which  $I_{ref}/M_{ref}$  is measured, the normalized intensities of the VO-P spectra (reported in wt%) of historical samples are  $\frac{I_s M_{ref}}{I_{ref} M_s} \times 100$  (horizontal axis of Fig. 4c).

These details have been specified (in red) in the experimental part of the manuscript (lines 94-98).

The horizontal error bars are very small and correspond to the size of the data point. This is because the sample mass and amplitude of the hf line are measured with good accuracy. This information has been added in the caption of Fig.4c (in red).

**Comment #5:** *The X/Y ratio model assumes a Gaussian lineshape (see SI page 7, line 83). What is the assumed linewidth, and how is it justified. Also, why is the assumption of a Gaussian lineshape valid ?*

**Response:** In such amorphous solids with complex compositions and heterogeneous micro/nano-structures, and which are likely to have undergone weathering phenomena over time, a distribution of hyperfine couplings is naturally expected. Such distributions around a central value are generally Gaussian. A Gaussian line shape has therefore been postulated in the model developed in the SI. However, it is important to note that the conclusions of the model are not affected if another line shape is assumed. The only important thing is that the line shape is the same for all samples in the corpus, which is a reasonable assumption. A sentence has been added in the SI.

**Comment #6:** *Do the spectral features for A-parallel and A-perpendicular occur at the same frequency offset in all samples ?*

**Response:** Yes, the ENDOR features occur at the same frequency offsets in all samples of the corpus (this can be qualitatively seen in Fig. S5). Except for sample Hum 1, for which these ENDOR features are not detected because of the very low bitumen content, this indicates that the hyperfine coupling to the bridging hydrogens of the porphyrin rings is independent of the bioorganic content of the mixture, and thus that VO-P complexes are little affected by this mixture.

**Comment #8:** *Line 231: The statement that the A-parallel 1H ENDOR peak broadens and weakens as more bioorganic matter is mixed with bitumen needs some supporting evidence. Were reference spectra recorded for this ? It is obvious that disorder in the hyperfine coupling will broaden and weaken the peak, but from where is it known that the mixing of bitumen with other substances will lead to this ?*

**Response:** The weakening of the intensity (due to the decrease of the bitumen content of the mixture) and the broadening (due to the disorder) of the parallel components of the ENDOR spectra are just an experimental fact. This cannot be verified simply by making reference samples (for example by mixing bitumen and various natural substances), as this presupposes that we know the details of the recipes of the Egyptian priests (which was kept secret), which is precisely one of the objective of our work.

**Comment #9:** Line 238: It is implied that protons more than 5-6 nm from the electron have zero electron-proton dipolar interactions. This is not correct. The interactions are small, but not zero.

**Response:** Your remark is absolutely correct, but what we mean is that the dipolar interaction has no effect on the matrix ENDOR line shape and intensity beyond a certain distance  $r$ , even if this interaction is not zero. Kevan et al. (1976) have modelled the matrix lines in disordered systems, whose line shapes obey the expression:

$$f(\nu) = N \int_0^{\pi/2} \int_a^{\infty} \frac{\cos^2 \theta \sin^2 \theta}{r^4} \left[ \frac{1}{\alpha^2 + [\nu - (q/r^3)]^2} + \frac{1}{\alpha^2 + [\nu + (q/r^3)]^2} \right] d\theta dr$$

With  $a$  the average size of the unpaired electron wave function. They showed that  $f(\nu)$  is almost independent of the electron-proton distance for  $r$  larger than a few nanometers.

Consequently, we have replaced “(limit for non-zero dipolar interaction)” by “(limit for an effect on the matrix line)” (line 247, in red).

**Comment #10:** Line 265: It is stated that VO-nP complexes are localized to the interface between bitumen aggregates and natural substances. Could it also be that VO-P from bitumen has been solubilized as VO-nP during processing and has migrated into the non-bitumen phase? How can this be excluded?

**Response:** Very good question, which we have been asking ourselves throughout this work. What is certain is that the vast majority of VO-Ps are intimately bonded to the asphaltene macromolecules of bitumen. This fraction is insoluble in organic solvents (but soluble in toluene). Thus, when all the organic components of the black matter of the historical samples are separated by treatments in n-alkanes and other solvents, only the insoluble asphaltene component precipitates, dragging with it the VO-Ps. In the historical samples, it is therefore highly doubtful that the VO-Ps can solubilize in the bioorganic component of the mixture. In a work in progress, we have recently measured electronic  $T_1$  and  $T_2$  for VO-Ps and  $C^0$  radicals in these samples. Relaxation times  $T_1$  and  $T_2$  of VO-Ps do not vary across the series of historical samples and are the same as in pure natural bitumen ( $T_1 \approx 1.5 \pm 0.5 \mu\text{s}$ ,  $T_2 \approx 0.7 \pm 0.1 \mu\text{s}$ ), showing that the VO-Ps always “see” an asphaltene environment, whatever the bioorganic content of the samples. If the VO-Ps were more or less solubilized in the bioorganic component, variations in  $T_1$  and  $T_2$  would be expected across the series and would be different from values measured in pure bitumen. For these chemical and physical reasons, we assume that the trans-metalation reaction (formation of VO-nPs) takes place at the interface between asphaltene (which provides vanadyl ions) and the natural substances (which provide oxygenated ligands). However, as the black matter was viscous during the preparation of the mummies, it is likely that the VO-nPs, once formed by complexation with oxygenated functions of the bioorganic molecules, can subsequently diffuse into the bioorganic component before complete solidification of the coating. As the study of VO-nP is not the subject of this manuscript, we have chosen not to weigh down the text with these details.

**Comment #11:** *In Eq.(3), it is not clear what  $A_{\lambda}(2)$  in the second term represents. Does the 2 indicate squaring or something else ?*

**Response:** This is probably a printing error on your side, because this symbol does not appear in the version available in Copernicus website. Equation 3 reads

$$v_{dq}^{\pm} = A \pm 2v_N + \frac{A^{(2)}}{(A/2) \pm v_N}$$

where  $A^{(2)}$  is the second order contribution to the hyperfine interaction, given in Eq. S8.

**Comment #12:** *Regarding the orientation of the  $^{14}\text{N}$  quadrupolar tensor, what the DFT calculations predict ?*

**Response:** It is rather difficult to answer this question because DFT calculations give different predictions. Gracheva et al (2016) used the ORCA code to predict the  $^{14}\text{N}$  ENDOR spectra for vanadyl porphyrins in crude oil. They obtained  $Q_{zz}$  in the porphyrin plane, however their calculated ENDOR spectra differ somewhat from the experimental spectrum, especially for the magnetic field setting in the porphyrin plane. Gourier et al. (2010) used the Gaussian code to predict the  $^{14}\text{N}$  HYSCORE spectrum of vanadyl tetraphenylporphyrin (VOTPP). They obtained  $Q_{zz}$  nearly parallel to the VO bond, with simulated spectra in fairly good agreement with experimental spectra for both parallel and perpendicular field settings. In the present work, the experimental spectra can be fitted correctly only if  $Q_{zz}$  is considered parallel to the VO bond, otherwise there is a factor of 2 between the value of  $Q_{zz}$  obtained by fitting the spectra and the values of  $Q$  measured from sq-dq correlation peaks for the perpendicular field setting.

**Comment #13:** *By assessing different  $^{14}\text{N}$  peaks in the HYSCORE spectra to different VO-P species, it is implicitly assumed that all four nitrogens in a porphyrin complex has the same coupling parameters. Is this assumption valid ?*

**Response:** This is an important question, which may not be fully answered at present. On the one hand it is known that there are many varieties of vanadyl geoporphyrins in oil and bitumen, so they can be expected to differ in their  $^{14}\text{N}$  hf coupling. On the other hand, VO-Ps with non-axial symmetry may exhibit different hf couplings for the four nitrogen ligands. So either we are in the presence of only one (or two) type(s) of VO-P with different  $^{14}\text{N}$  hf couplings, or we are in the presence of 4 different types of VO-Ps which are distinguished by the mean values of their  $^{14}\text{N}$  hf coupling. The facts that the VO-P1 coupling is found in all 4 samples, while VO-P2 is found in only 3 samples, and that the 4 samples have either VO-P3 or VO-P4, but not both at the same time, favor the hypothesis of different types of VO-Ps rather than different types of nitrogen in a single VO-P. This is the choice we made.

#### Other notes

- *Line 69: It is unclear what a "hindered" hyperfine interaction is. Maybe "unresolved" hyperfine interaction is the intended meaning.* Right. This is now corrected in the manuscript.
- *Line 75: "Than in" → "as in"* Thank you. This is now corrected in the manuscript.
- *Figure S6: "chlorophyle" → "chlorophyll"* Yes, thank you. This is now corrected in the manuscript.

- *In general, // should be replaced by || to indicate "parallel".* It depends. We have always used this notation and it has always been accepted by reviewers and editors.
- *Line 104: Pulse EPR was probably done on an E580, not a E500 spectrometer.* Yes, thank you. This is now corrected in the manuscript.
- *Figure 3d: the three colors for the three wave packets indicating the three microwave pulses should be the same; different colors visually implies different frequencies.* The three colors indicate that these wave packets have a different role in the mechanism, but we can see that they have the same wavelength. In the case of ENDOR (Fig.3c), the rf line clearly has a wavelength larger than the microwave.
- *Line185: In cw ENDOR, the RF field doesn't have to be saturating.* We have removed "saturating" from the text. We used this word because the rf field changes the population of nuclear spin states.
- *Line 388: What is meant by "hindered" ? "hidden" ?* Yes, thank you for the remark.