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1 Rapid and Complete Prediction of Alizarin in Solution by Combining 2 Experimental Data with Computational Methods

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11 ABSTRACT

12 In the field of cultural heritage, understanding the nature of pigments and dyes is of great importance for
13 conservation issues. Among the many natural dyes used in paintings or textiles, madder was one of the most
14 important sources of red color at the time and was present in a wide variety of objects. However, due to
15 extraction challenges, impurities, and high costs, a lot of information are not easily available. In this
16 publication we designed a computational protocol able to reproduce the spectroscopic properties of two
17 dyes, alizarin and alizarin red S. This framework allows us to compute both Ultraviolet-Visible absorption
18 spectra and Nuclear Magnetic Resonance (NMR) with good accuracy as well as reproduce with precision the
19 CIELAB color. We have also explored different types of interactions that impact these properties, notably the
20 solvation effect. We found that microsolvation is sufficient to reproduce the experimental measurements
21 made in water. The high accuracy of the computation method makes this technique particularly promising
22 for a non-destructive study of dyes on works of art and the preservation of cultural heritage.

23

24 INTRODUCTION

25 The analytical investigation of dyes is considered of utmost importance in the field of cultural heritage.
26 Indeed, through the identification of the corresponding dye, we are often able to gain insight into several
27 important aspects: the characterization of the dye and the artist's techniques, the degradation pathways,
28 the preservation state of the artifact, the dating and the determination of the provenance of objects [1–4].
29 Furthermore, it provides precious details to plan correct restoration, conservation, and display strategy in
30 museums and art galleries.

31 Both traditional and cutting-edge techniques such as ultraviolet-visible (UV-Vis), infrared (IR), and Raman
32 spectroscopies, as well as nuclear magnetic resonance (NMR), mass spectrometry (MS), liquid
33 chromatography, Fiber Optics Reflectance Spectra (FORS), X-Ray diffraction (XRD) or hyperspectral
34 spectroscopy have been reported for the analytical investigation of dyes [2,5–14]. While the accuracy and
35 sensitivity of these techniques have allowed researchers to gain a huge amount of insight into these
36 systems, most of the technologies are sampling-based and need complex dye extraction and chemical
37 preparation methods. Moreover, sample collection, preparation, and chemical analysis are often challenging
38 due to material complexity, limited sample sizes, dye degradation, and lack of appropriate standards needed
39 for UV-Vis detection [8,15–19]. Therefore, the identification of historical dyes is still one of the most
40 challenging tasks in the realm of heritage preservation. In this context, computational methods have
41 emerged as an appealing tool to decipher complex experimental data [20]. These theoretical methods aid in
42 the interpretation of individual contributions to the experimental spectrum. Unfortunately, their application

43 in the field of cultural heritage is not as widespread as other fields of research (such as catalysis,
44 astrochemistry, electrochemistry, etc).

45 Representing one of the most famous natural dyes since antiquity, madder was selected as the focus of this
46 study [21–23]. From the roots of this plant, around 36 red colorants derivating from the anthraquinone
47 family can be extracted in various quantities. We will focus here on alizarin, one of the main constituents of
48 madder [24,25].

49 In order to study the associated dyes, the combination of NMR with UV-Vis is often used in the heritage
50 science field because of their complementary nature. While UV-Vis is good at characterizing the
51 environmental effect of the dye or its degradation (color fading), NMR can differentiate analog molecules. It
52 is also very precise in attesting chemical modifications, notably when lake or pigment structures are created
53 by association of a colored dye with a metallic ion.

54 For the computation of spectroscopic properties, density functional theory (DFT) and Time Dependent-DFT
55 are often the methods of choice due to their reasonable costs and good precision. In fact, there have been
56 many reports regarding the prediction of Alizarin color and properties using these methods [26–37]. Still, the
57 role of the environment is not clearly understood or deciphered. Furthermore, some key properties like
58 reproducing the UV-Vis or NMR spectra are not always present.

59 When examining molecules using spectroscopic analysis, a significant portion of their observable
60 characteristics displays varying degrees of sensitivity to the surrounding environment (solvent, pH, binding
61 partners, mordant, substrate, temperature, light, etc) [38,39]. This influence of the solvent on spectroscopic
62 properties arises from multiple factors such as the polarity of the solvent or the presence of hydrogen
63 bonds. It is therefore crucial to find the optimal solvation model in order to correctly predict the UV-Vis
64 spectrum or other properties of these organic molecules.

65 In this study, our attention will be directed toward the impact of solvents on a precious dye, alizarin. The aim
66 is to introduce a holistic computational protocol designed to predict color and chemical properties,
67 developed through a collaborative effort with experimental data. The protocol integrates multiple facets,
68 including the exploration of conformers and deprotonated forms, effective selection of optimal solvation
69 models based on the solute-solvent system of study (implicit, microsolvated, or multilevel), prediction of UV-
70 Vis spectrum, color prediction with CIELAB coordinates and ¹³C and ¹H NMR shift predictions. This protocol
71 will be applied to two dyes: alizarin and alizarin red S.

72

73 **1. MATERIALS & METHODS**

74

75 **2.1. EXPERIMENTAL METHOD**

76 *UV Spectrum*

77 Alizarin (97% purity) was purchased from Sigma Aldrich and used without further purification. Alizarin red S
78 pure (sodium alizarinsulfonate) was purchased from Thermoficher.

79 10 mg of alizarin were dissolved in 40 mL of deionized water (MilliQ). The alizarin solution was then heated
80 for 2 hours at 90°C, and filtered using a Buchner funnel. 5mg of alizarin red S was added to 80 mL of distilled
81 water and stirred for 10min. The obtained solution was used directly without heating or filtering. Then, the
82 pH was adjusted using either hydrochloric acid (HCl, 0.1M) or sodium hydroxide (NaOH, 1M). Three pH
83 values were considered for alizarin (pKa₁=5.5 and pKa₂=10) : 3, 8, and 12. For alizarin red S (pKa₁=5.8 and
84 pKa₂=10.8), the following pH were used: 2, 7 and 12 [40,41].

85 The UV-Vis spectra were measured directly after each preparation. It was done using an Ocean Optics Flame
86 spectrometer (Model: FLMS00699) with a wavelength range from 300 to 850 nm. The integration time for
87 each measurement ranged from 5 milliseconds to 10 milliseconds. The light source used was a Deuterium-
88 Tungsten Halogen lamp covering the range of 300 nm to 850 nm. The blank was done using deionized water.
89 This light source was coupled to the spectrometer using 400 μ m diameter optical fibers and positioned
90 through a 1 cm quartz cuvette holder. The acquisition of spectra was facilitated by the Ocean View software.

91 *NMR Spectrum*

92 NMR spectra were acquired with a Bruker Advance Neo 500 spectrometer at the frequency of 500.07 MHz
93 for ^1H and 125.76 MHz for ^{13}C equipped with a 5 mm double resonance broadband prodigy probe use
94 nitrogen-cooled RF coils and preamplifiers. Chemical shifts were calibrated using the Tetramethylsilane (0
95 ppm). For ^1H , we used a one 90° pulse with 64 scans and with a recycling time of 1s. For ^{13}C , we used a one
96 90° pulse with a ^1H decoupling waltz16 with 4096 scans and with a recycling time D1 of 2s. The ^1H and ^{13}C
97 spectra were recorded in exactly the same conditions at room temperature and processed with a zero-filling
98 factor of 2 and without an exponential decay. 5mg of either alizarin or alizarin red S were dissolved in 0.5mL
99 of acetone- D_6 . The ^1H and ^{13}C NMR spectra were also measured for alizarin red S in D_2O as it was more
100 soluble than alizarin.

101

102 **2.2. COMPUTATIONNAL METHOD**

103 All calculations were carried out using the version 5.0.3 of the ORCA quantum chemistry program package
104 and the version 6.5.1 of xTB [42,43].

105 *Optimization Procedure*

106 Ground and excited states properties were computed using density functional theory (DFT) and its time-
107 dependent (TD-DFT) extension [44,45]. Optimized Geometries and Hessians were obtained using the PBE0
108 functional [46] complemented by the D3(BJ) dispersion correction [47,48]. Tight thresholds were employed
109 for the self-consistent field (SCF) and optimization (tightSCF and tightopt keywords in ORCA nomenclature)
110 [47,49]. The triple zeta def2-TZVP basis set, coupled with matching auxiliary basis sets, was consistently
111 employed throughout all the computations [47,49,50]. All the optimized geometries can be found in the
112 supplementary data 14.

113

114 *Conformer and deprotonated Forms Search*

115 Using Grimme's open-source Conformer-Rotamer Ensemble Sampling Tool (CREST), which relies on the xTB
116 method, the exploration for deprotonated forms and conformer for the fully protonated form was
117 conducted [43,51]. The geometries of the generated conformer or deprotonated forms were then subjected
118 to optimization using the PBE0 functional. Finally, their single point energy (SPE) were obtained by using
119 DLPNO-CCSD(T) computations with def2-TZVP basis sets with matching auxiliary basis sets and CPCM (water)
120 [52]. Their SPE energies are then compared for each deprotonated or conformer forms to identify the most
121 energetically stable forms (the one with the lowest SPE energy). By comparing the SPE energies, we can
122 calculate the Boltzmann population distribution of each form. The identified stable forms correspond to the
123 highest Boltzmann distribution. Those forms are dominant and are therefore selected for the following
124 computations.

125 *Solvation Models*

126 Different solvation models were evaluated for UV-Vis and NMR spectra prediction. These models included
127 the following:

128 -In vacuo: the molecule is studied in the absence of any solvent.

129 -Implicit solvation (water or acetone) using the linear response Conductor-like Polarizable Continuum Model
130 (C-PCM) [53]. No solvents appear but it treats the solvent as a continuous medium surrounding the solute.
131 This model accounts for the electrostatic and non-polar interactions between the solute and the solvent
132 without explicitly representing individual solvent molecules. Their bulk properties are replaced by some
133 macroscopic quantities (dielectric constant or solvent radius).

134 -A combined approach involving both implicit and explicit water molecules (either 1 water molecule per
135 hydroxyl (OH) group or 3 water molecules per OH group). The water molecules were positioned to make
136 hydrogen bonds with each hydroxyl group.

137 -Furthermore, a hybrid approach called Quantum Mechanics/Quantum Mechanics 2 (QM/QM2) [54–56]
138 calculations were conducted by engaging between 70-100 water molecules. It combines quantum mechanics
139 (QM) for a region of interest (here the dye molecule) and the semi-empirical GFN2-xTB method [43,51] for
140 the rest of the system (here the surrounding water molecules). The optimization was accomplished using the
141 multiscale module in ORCA [42,57] together with the QM/QM2 ONIOM procedure. This allows detailed
142 treatment for the electronic structure of the solute while considering the surrounding solvent at a less
143 computationally expensive level. To consider the solvent effect, we also performed a Quantum Molecular
144 Dynamics simulation of 25,000 steps with a 0.5 fs time step (other parameters selected as default such as a
145 NHC (Nosé–Hoover chains) thermostat of 300K) [58]. An ellipsoid repulsive wall was added to keep the
146 water molecules close to the substrate during the MS. From this simulation, 50 snapshots were selected,
147 each 200 steps starting at 800. They were then optimized and the mean Vertical Energy (VE) of these
148 snapshots calculated.

149 *UV-Vis Spectrum computation*

150 For Time-Dependent Density Functional Theory (TD-DFT) calculations concerning the UV-Vis spectrum, the
151 B2PLYP functional was used with the resolution of identity method (RI) [30,59,60]. This choice is justified by
152 previous theoretical benchmarks and preliminary calculations (see supplementary data 2) [61,62]. The UV-
153 Vis VE calculations were done with the first 15 roots. The experimental spectra of the dyes studied (alizarin
154 and alizarin red S) has only one band on the visible region. In all calculations, only the first excited state was
155 considered for the VE calculation, as it can be compared to the experimental maximum absorption of the
156 Visible region of the UV-Vis spectrum. The topology and form of the experimental spectra are due to
157 vibrational and solvent effects which are not present when calculating the VE but will be accounted using
158 another calculation. The color of the compound depends on the form of the spectra, it is therefore necessary
159 to take it into account.

160 The vibrational contributions, including Herzberg–Teller effects [63–65], was accomplished using the Excited
161 State Dynamics (ESD) module within ORCA [66]. Within this framework, the Adiabatic Hessian (AH) and
162 Vertical Gradient (VG) approaches were used. As AH is a more complete and accurate method, computed
163 spectra within the main text will be performed using this scheme [67]. VG spectra can be found in
164 supplementary data 5. The AH calculations require the geometry of both Ground State (*GS*) and the first
165 singlet excited state (*S*₁) geometries and frequencies, the VG ones only required the *GS* parameters, all of
166 them computed at the DFT PBE0 level + CPCM. The VE energy and transition dipole moments were
167 computed using the most accurate values *i.e.* RI-B2PLYP together with the appropriate solvation scheme (3
168 water molecules per OH). The linewidth was created using a Gaussian distribution and was fine-tuned to
169 align with the Full Width at Half Maximum (FWHM) parameter of the experimental spectra, which depends
170 on the concentration and other experimental parameters. To reduce the impact of experimental parameters
171 like concentration, the computed and experimental spectra were normalized. This allows a direct
172 comparison between the two spectra.

173 The CIELAB color coordinates was calculated for the UV-Vis spectra with an in-house script with illuminant
174 D65 [68–70]. To quantify the quality of the color prediction, the color difference was quantified with ΔE_{2000}
175 which is the latest ISO standard by CIE [71,72].

176 *NMR Spectrum computation*

177 Geometry optimization of tetramethylsilane (TMS), alizarin, alizarin red S molecules was conducted within
178 vacuo, an implicit acetone or water solvent environment using the CPCM method or with explicit acetone or
179 water molecules as described before. Subsequent computation of ^1H -NMR and ^{13}C -NMR shielding employed
180 the revTPSS functional with D4 correction [73–75]. Basis set pcSseg-3 was selected for these calculations.
181 Next, the isotropic shifts were used to compute the shifts that are comparable to the experimental by using
182 this formula:

$$\sigma_{\text{calculated}} = \sigma_{\text{TMS}} - \sigma_{\text{targetmolecule}}$$

183 A summary of the computational protocol is summarized in a flow chart in the supplementary data 1.

184

185 2. RESULTS AND DISCUSSION

186

187 Fig.1 presents the structure of the studied molecules: alizarin (later referred as H_2A) and alizarin red S (later
188 referred as H_2ARS). Both alizarin and alizarin red S have two acidic protons which can be subject to
189 deprotonation depending on the solutions' pH. Alizarin (alizarin Red S) has $\text{pK}_{a1}=5.5$ and $\text{pK}_{a2}=10$ ($\text{pK}_{a1}=5.8$
190 and $\text{pK}_{a2}=10.8$) respectively. Therefore, in this study, we have chosen the following pH for each form: pH=3,
191 pH=8, and pH=12 for alizarin and pH=2, pH=7 and pH=12 for alizarin red S [40,41].

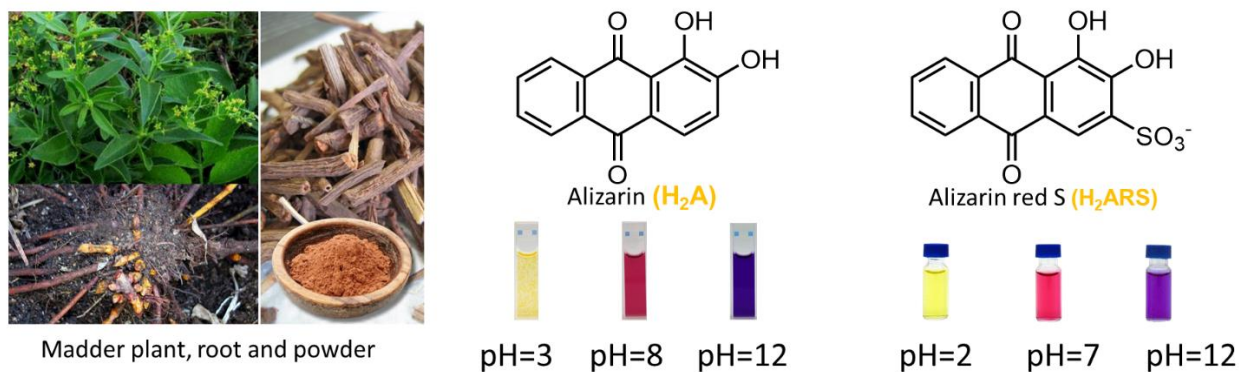


Fig. 1. (Left) Madder plant, its roots and powder. (Middle) Chemical structure of alizarin, abbreviated as H_2A and its alternative more soluble in water, alizarin red S (right), abbreviated as H_2ARS . The color of each molecule for different pH level are depicted below each structure.

192 This study aims to establish a reliable computational protocol in order to reproduce their spectroscopic
193 properties (UV-Vis spectra, color and NMR spectra) and provide us with relevant chemical information.

194

195

196 3.1. ALIZARIN

197

198 3.1.1. UV-Vis spectra

199

200 3.1.1.1. Conformer and deprotonated forms search

201 The initial step involves exploring the conformer and deprotonated forms of alizarin for each pH range in
202 order to identify the most stable forms.

203 In the protonated form (neutral) at pH=3, various studies have addressed the presence of different
204 conformers [36,76]. We first generated the conformer of the neutral protonated form of alizarin and
205 computed their Single Point Energy (SPE). The one with the lowest SPE energy after optimization is the most
206 stable and is taken as a reference. The other conformer forms are listed by energy (in kcal/mol) with respect
207 to the most stable form in Fig 2. We have found that the dominant form is the one with the hydrogen of
208 both the α and β -hydroxyl groups making hydrogen bonds with the neighboring oxygen. It will be later
209 referred to as H_2A . Furthermore, for the ground state, the forms with the proton transfer on the ketone as
210 reported in previous publications are much higher in energy (14.07 kcal/mol respective to H_2A), meaning
211 that in the ground state, there is likely no proton transfer for the neutral form in water. The remaining
212 conformer forms do not exist in solution as they are higher in energy than the most stable one.

213 For the mono-deprotonated form (monoanionic) at pH=8, two potential forms are possible due to the
214 presence of two deprotonation sites (see Fig. 2). However, by comparing the computed SPE, the most stable
215 configuration is the one with the β -hydroxyl being deprotonated. The other form with the α hydroxyl
216 deprotonated is higher in energy by 0.9 kcal/mol. This corresponds to the Boltzmann distribution of 82% in
217 favor of the most stable form. This can be explained by the formation of intramolecular hydrogen bonds
218 between the ketone and the α hydroxyl group, making it more stable and therefore less favorable to
219 deprotonation than the β -hydroxyl group. Furthermore, the hydrogen in the β position can be more
220 accessible to the surrounding solvent, making it more susceptible to deprotonation. The most stable form
221 will be used to do further calculations as it will be the most dominant. We will denote it as HA^- .

222 For the di-deprotonated (dianionic) form at pH=12, there is only one stable form without other conformers.
223 It will be denoted as A^{2-} .

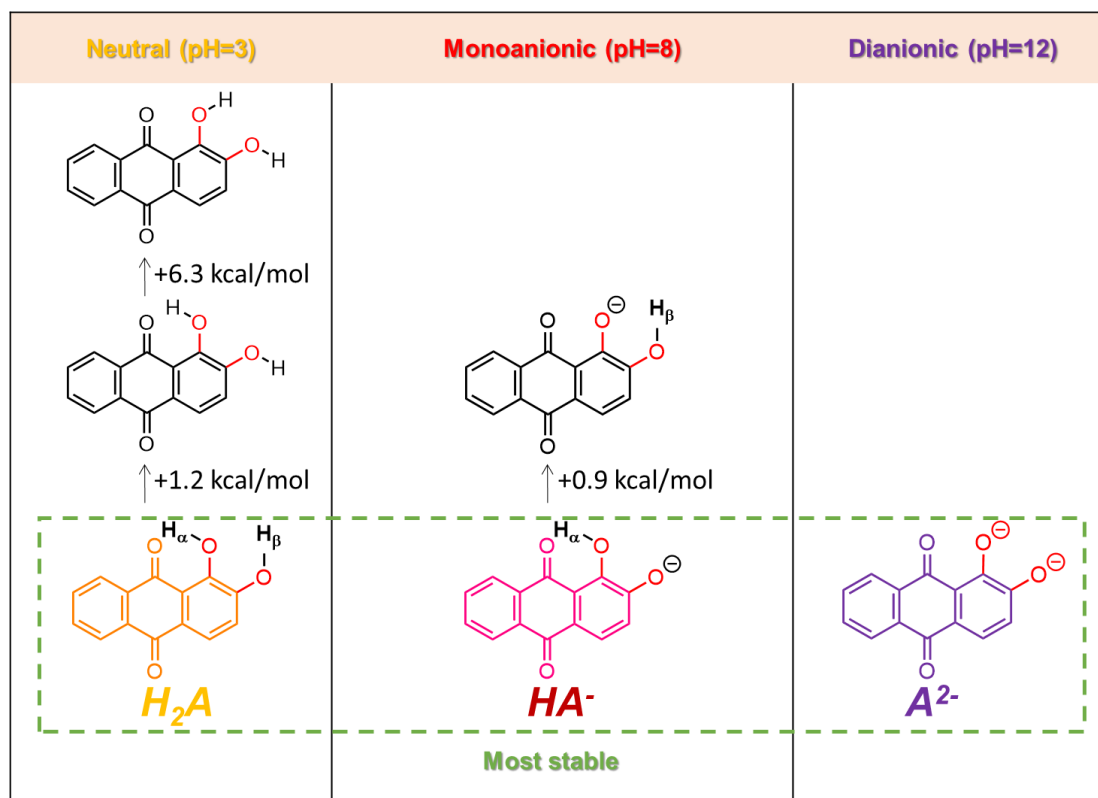


Fig. 2. Alizarin rotamers and deprotonated forms for each pH range and their relative energies. For the fully protonated form at pH=3. The geometries are sorted from the most stable form to the less stable form. The most stable forms are denoted H_2A (neutral form), HA^- (monoanionic form), and A^{2-} (dianionic form).

224

3.1.1.2. Vertical energy and choice of solvation model

225

In computational chemistry, the choice of solvation model is critical as it determines the accuracy of the predicted UV-Vis spectra [77]. In fact, the geometry of the molecules can be influenced by the surrounding solvent via specific interactions like hydrogen bonds, ion pairs, π -interactions, etc. Here, we have tested different solvation models to compute the VE between the electronic GS and S_1 and compare it to the maximum absorption of the experimental visible spectra. The calculations were first done in vacuo, then with implicit water, a combined implicit and explicit, and finally with a multi-level approach such as QM/QM2 in combination with Molecular Dynamics. All computational details of those solvation models are outlined in the *Methods* section. Fig.3 presents a condensed view of the different models used in this study and the associated results for the alizarin forms H_2A , HA^- and A^{2-} (Additional information can be found in supplementary data 3).

234

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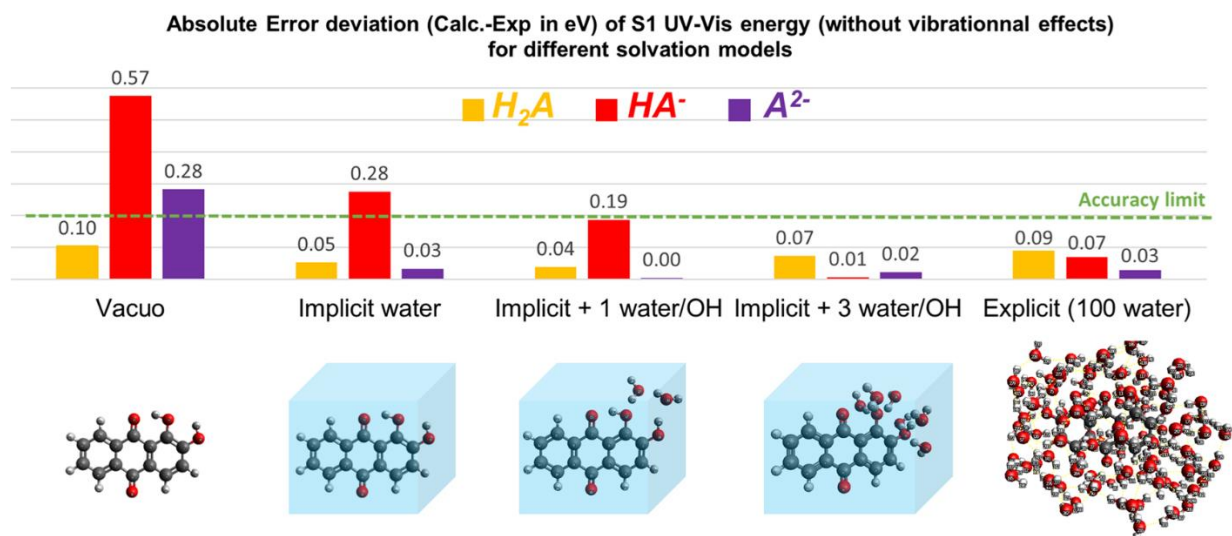


Fig. 3. (Top) Absolute energy difference between the calculated VE and the experimental absorption energy for the different solvation models of alizarin. The yellow stands for the H_2A form, the red for HA^- one and the purple for A^{2-} . The accuracy threshold is fixed at 0.2 eV according to the literature. (Bottom) Different solvation models were used. From left to right: alizarin in vacuo, implicit water, implicit with 1 water per hydroxy group, implicit with 3 water per hydroxy group, QM/QM2 with 100 explicit water (over 50 snapshots).

236 We first did the calculations in vacuo, without any solvent as a study case. However, it does not provide
 237 insights into the effects of a surrounding medium, which is often crucial for realistic scenarios. Here, it was
 238 observed that for the neutral form, the vacuo model is accurate enough (the error deviation falls below the
 239 0.2eV accuracy limit which is the standard in the literature). However, for the deprotonated forms
 240 (monoanionic HA^- or dianionic A^{2-}), the vacuo model is not accurate enough (error deviation of 0.57eV for
 241 HA^- and 0.28eV for A^{2-}). It is therefore necessary to include solvent effects for these forms.

242 A first approximation of the solvent effect can be done by using an implicit model. For alizarin, implicit
 243 solvation gives better agreement with experimental data by reducing the error by at least half (it falls to
 244 0.05eV for H_2A , 0.28eV for HA^- and 0.03eV for A^{2-}). It can be used to test different solvent environments
 245 while being computationally efficient. This approach is useful for the case where there is no apparent
 246 interaction between the solvent and the dye, like the neutral form H_2A in water or for apolar solvents.
 247 However, the HA^- form is still above the accuracy limit (with 0.28 eV error deviation) in implicit calculation.
 248 Therefore, we need to account for specific solvent effects. For those forms, the model can be refined by
 249 adding some explicit solvent molecules near the hydroxyl sites. Explicit water molecules (either 1 or 3) per
 250 hydroxy group were tested. This considerably reduces the calculated and experimental energy difference for
 251 the deprotonated forms. Among them, the best model is the one with 3 explicit water molecules near the
 252 OH sites (error deviation of 0.01 eV for HA^- and 0.02 eV for A^{2-}). This shows that for the deprotonated forms,
 253 solvent interactions like hydrogen bonds are mandatory to accurately model the absorption energy.
 254 Therefore, this combined model can be a good compromise to accurately predict the UV-Vis energies for a
 255 low computational cost. It can be used when there is a specific solute-solvent interaction.

256
 257 Finally, the last model tested was QM/QM2 with 100 explicit water which represents a theoretical best
 258 estimate for our protocol. For each form, a quantum molecular dynamic was performed followed by
 259 computing the VE of 50 snapshots. Here, it was observed that across all the forms, the QM/QM2 results
 260 consistently fall below the 0.2 eV accuracy threshold (0.09 eV for H_2A , 0.07 eV for HA^- and 0.03 eV for A^{2-}).
 261 This implies that QM/QM2 is a reliable method for predicting the properties of new molecules for which the
 262 solute-solvent interactions are predominant but requires a much higher computational cost, at least 50

263 times higher. More information about the MD and the selected snapshots can be found in supplementary
 264 data 4.

265
 266 From the previous paragraphs, it is clear that the accuracy of the VE is tightly bound to how the
 267 environmental effects are modeled. As water is a highly polar solvent and involved in multiple inter-
 268 molecular interactions, one should expect that explicit molecules have to be added. Interestingly, for some
 269 forms, implicit solvation is accurate enough, notably for A^{2-} , where two charged oxygens are present.
 270 However, only the microsolvation with 3 added water molecules per OH is sufficient to get close to the
 271 experimental measurements for all forms. This model also gives similar results in comparison to a full explicit
 272 model, allowing us to ensure that it is reliable and transferable to similar dyes.

273
 274
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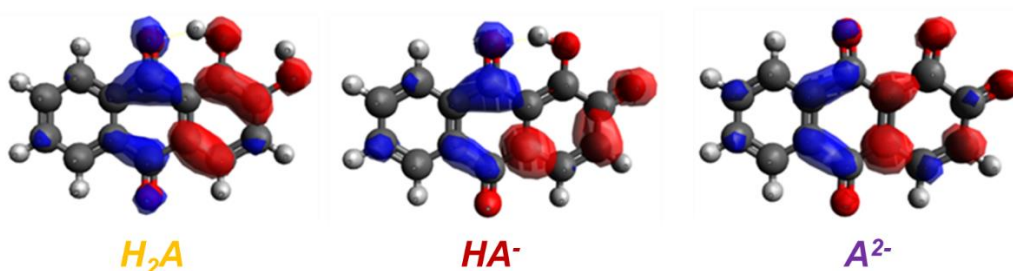
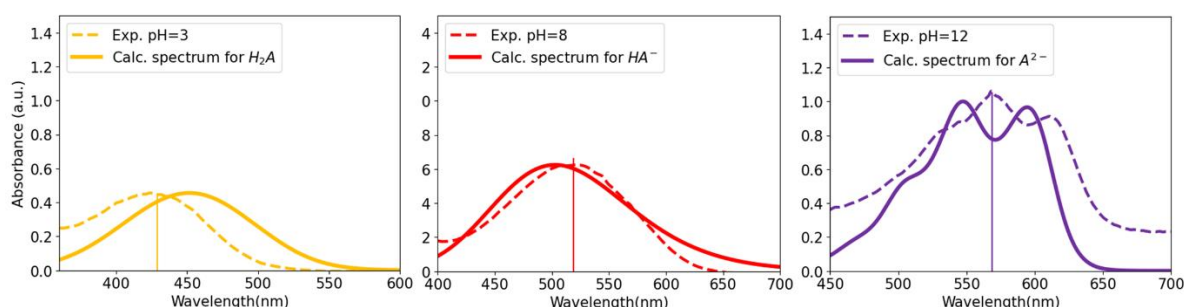


Fig. 4. Difference in electronic densities for the $GS \rightarrow S_1$ transition for alizarin for different protonation states. The blue orbital represents a decrease in electronic density. The red one represents an increase in electronic density. The isovalue is 0.02 a.u.

276 To further delve into the nature of electronic transitions, the difference in electronic densities has been
 277 calculated for the transition $GS \rightarrow S_1$ (which corresponds to the absorption in the visible part of the UV-Vis
 278 spectrum). The result for each alizarin form is depicted in Fig.4.

279 For the neutral form H_2A , the transition involves a combined $n \rightarrow \pi^*$ (owing to the presence of oxygen) and $\pi \rightarrow$
 280 π^* transition, with a more pronounced $\pi \rightarrow \pi^*$ character. As we progress to the deprotonated form, HA^- and
 281 A^{2-} , the transition nature shifts more towards $\pi \rightarrow \pi^*$ transition rather than $n \rightarrow \pi^*$ as the deprotonated
 282 hydroxy groups become part of the conjugated system.

283 3.1.1.3. Computed absorption spectra and CIELAB color



Form	Experimental (normalized)				Calculated (normalized)				ΔE_{2000}
	L*	a*	b*	color	L*	a*	b*	color	
H_2A	99.0	-13.4	68.4		90.7	6.9	80.4		13.3
HA^-	67.5	53.3	0.9		59.1	45.5	3.2		7.5
A^{2-}	54.1	51.9	-50.0		57.0	61.7	-53.6		4.1

Fig. 5. (Top) The calculated and experimental UV-Vis spectra for alizarin at different pH. The dotted line is the experimental one. The

full lines are the calculated spectrum with the Adiabatic Hessian (AH) method. The vertical lines correspond to the computed VE. (Bottom) Predicted and computed colors of different alizarin forms with CIELAB coordinates (L^* , a^* , b^*) and the calculated color variation between computed and experimental color coordinates with ΔE_{2000} .

284 The VE alone is not enough to predict the full spectrum because it does not consider vibrational effects
285 which can be responsible for the UV-Vis spectra topology (shape). In Fig.5. we have computed the
286 absorption spectrum of alizarin at different pH and compared them with their experimental UV-Vis spectra.
287 First, if we look at the different pH forms of alizarin we can see that their UV-Vis spectra shape and color
288 differ. When the pH increases, we see a red shift of the spectra together with a hyperchromic shift (increase
289 in absorption intensity). For pH=3, the spectrum has a maximum absorption peak at 430nm, resulting in a
290 yellow solution. For pH=8, the solution turns red, with the maximum absorption at 530 nm. For pH=12, the
291 solution takes a violet hue, with a maximum peak at 570 nm and two shoulders at 530 nm and 610 nm.
292 These additional features could be attributed to several electronic transitions or a combination of electronic
293 and vibrational effects. To discern the nature of each transition, we used the Adiabatic Hessian method (AH).
294 The details of the computation can be found in the *Methods* section.

295 Overall, the computed spectra with AH are in good agreement with experimental data for both the
296 maximum and topology, in particular for HA^- and A^{2-} . For H_2A , the computed spectrum is shifted by about (27
297 nm) but it can be explained by the low solubility of the neutral form in water, preventing us from having a
298 reliable UV-Vis spectrum.

299 The A^{2-} replicates the experimental topology with three distinct shoulders. The VE corresponds to one
300 electronic transition from $GS \rightarrow S_1$. The middle peak is therefore attributed to one electronic transition, while
301 the shoulders around 540 nm and 610 nm result from vibronic effects.

302

303 While the absorption spectra are really useful in spectroscopic studies, in the cultural heritage or dyeing
304 field, the color of the molecule is the key property. In order to gain access to this information we can convert
305 the UV-Vis spectra to color CIELAB coordinates. CIELAB color space is a color model introduced by the
306 Commission Internationale de l'Eclairage (International Commission on Illumination or CIE) to provide a
307 uniform and consistent representation of colors [69]. It is designed to be independent of devices and
308 illuminants, making it useful for comparing colors across different conditions. Here, we will use it to compare
309 the computed and experimental color. The colors are expressed in $L^*a^*b^*$ coordinates. L stands for
310 Lightness and corresponds to the perceived brightness of the color. The values range from 0 (black) to 100
311 (white). The a^* stands for the position on the red(positive) to green (negative). b^* stands for blue (negative)
312 to yellow (positive). The CIELAB color space is represented in supplementary data 6. The bottom part of Fig.5
313 illustrates the predicted and experimental along with their respective CIELAB coordinates. ΔE_{2000} is the latest
314 standard created by the CIE and quantifies the color difference. E stands for "Empfindung" which is the
315 German word for sensation and 2000 stands for the version of standard (older versions exist like ΔE_{76} or ΔE_{94})
316 [68,71]. The standard perception ranges as follows:

- 317
- 318 • ≤ 1.0 : Not perceptible by the human eye
 - 319 • 1-2: Perceptible through close observation
 - 320 • 2-10: Perceptible at a glance
 - 321 • 11-49: Colors are more similar than the opposite
 - 322 • 100: Colors are exactly the opposite

323 The computed color closely resembles the experimental ones. A higher $\Delta E_{2000}=13.3$ is observed for the H_2A
324 form and HA^- ($\Delta E_{2000}=7.57$), possibly attributed to the lower solubility of alizarin in water, where a non-
325 negligible precipitate can be observed. In contrast, for A^{2-} which is more soluble in water, its computed color
326 is similar to the experimental ($\Delta E_{2000}=4.1$). That means that the color is similar but the difference can be

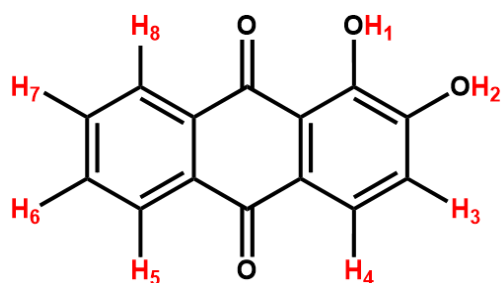
327 perceptible to the human eye at a glance. While the CIELAB colors are predominantly used for non-liquid
328 systems, our approach shows that computations can be modeled with a reasonably good accuracy.

329 3.1.2. NMR shift prediction

330 Another key property of chemical systems is their NMR spectra. It is often used to decipher the structure of
331 molecules in a complex environment, like pigments used in paintings, which are composed of a dye
332 complexed with a metallic cation. Our protocol will aim at reproducing with good accuracy the experimental
333 peaks.

334 Here, we present a study case with alizarin. The computed ^1H shift values were compared to the
335 experimental spectra of alizarin in acetone- D_6 (chosen because alizarin is not soluble in water) in Table 1. We
336 tested the influence of the solvation model with implicit and 3 explicit acetones following the UV spectrum
337 protocol. The calculated ^1H NMR shift differs by less than 0.16 ppm on average with respect to the
338 experimental one for aromatic hydrogens in implicit acetone and 0.22 ppm on average for explicit and
339 implicit acetone. This suggests a good level of accuracy for the prediction of proton chemical shifts for both
340 solvation models. The prediction of the ^1H shifts for hydrogens H3, H4, H6, and H7 are better with implicit
341 than explicit. However, it is noted that the accuracy is worse when dealing with the labile hydrogens in the
342 hydroxy (-OH) group (H1 and H2) with implicit solvation. Labile hydrogen is prone to exchange with the
343 surrounding solvents, it is therefore necessary to add explicit acetones near the hydroxy groups. For labile
344 hydrogens, the explicit solvation model yields better accuracy. Furthermore, H5-H8 are better with explicit
345 than implicit models. Explicit solvation is therefore more suited for labile hydrogens. The experimental
346 spectra are depicted in supplementary data 7, along with its attribution, which was done by confronting the
347 computed shifts.

Table 1. Computed and experimental ^1H chemical shift of alizarin in acetone- D_6 with the respective error deviation in ppm or % for implicit or implicit and explicit solvation model.



Attribution	Exp. (ppm)	Calc. (ppm)		Calc.-error (ppm)		Calc.-error (%)	
	In acetone- D_6	Implicit	Explicit	Implicit	Explicit	Implicit	Explicit
1	12.8	15.1	11.5	2.3	-1.3	18.2	10.2
2	9.3	7.2	10.0	-2.1	0.6	22.5	7.0
3	7.3	7.4	7.6	0.1	0.2	1.3	3.4
4	7.8	8.0	8.2	0.2	0.4	3.0	5.4
5	8.3	8.5	8.5	0.2	0.1	2.2	1.6
6	8.0	8.1	8.1	0.1	0.1	1.6	1.9
7	8.0	8.0	8.2	0.1	0.2	1.0	2.7
8	8.3	8.5	8.4	0.3	0.1	3.0	1.5

348

349 3.1.3. Experimental uncertainty due to solubility

350 As raised multiple times in this part, alizarin has a poor solubility in water. The color of alizarin in solution is
351 highly sensitive to experimental conditions such as concentration, pH and temperature. The maximum
352 absorption remains constant but the shape can potentially alter. Consequently, the measured color may vary
353 and a higher ΔE can be calculated with respect to the computed color. To address this experimental
354 obstacle, we opted for its alternative, alizarin red S which exhibits greater solubility.

355

356 3.2. ALIZARIN RED S

357

358 As we have seen, the limited solubility of alizarin in water can pose challenges when measuring their
359 experimental UV-Vis and NMR spectra due to the potential formation of precipitates. To address this
360 concern, a more soluble analogue, alizarin red S, has been studied.

361

362 3.2.1. Conformer and deprotonated forms search

363 For the acidic form (pH=2) of alizarin red S, different conformer forms can exist. We computed the energies
364 of the possible conformer and sorted them in Fig. 6. Like alizarin, we do not observe any proton transfer for
365 the ground state. The most stable form shows a hydrogen on the α position pointing towards the ketone,
366 making a hydrogen bond, while the β -hydrogen is pointing on the opposite side towards the negatively
367 charged sulfonic group. It will be noted as H_2ARS . The other conformer forms do not exist as they are much
368 higher in energy (hence low Boltzmann distribution).

For the mono-deprotonated form (pH=7), two possible forms can exist (see Fig.6). The comparison between energies point out that the β -hydroxyl deprotonated is the most stable configuration, the α -hydroxyl making a hydrogen bond with the ketone and is less susceptible to deprotonation. This form will be noted as $HARS^-$. For the di-deprotonated form (pH=12), there is only one form that will be denoted as ARS^{2-} .

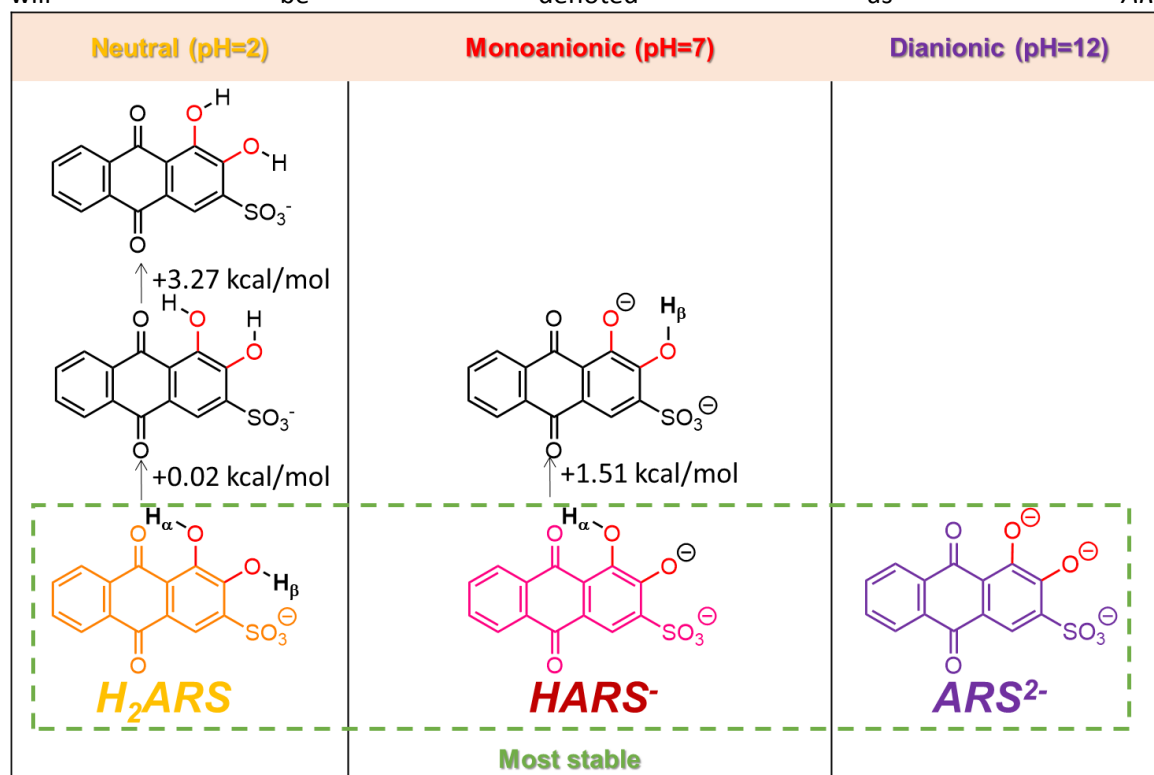
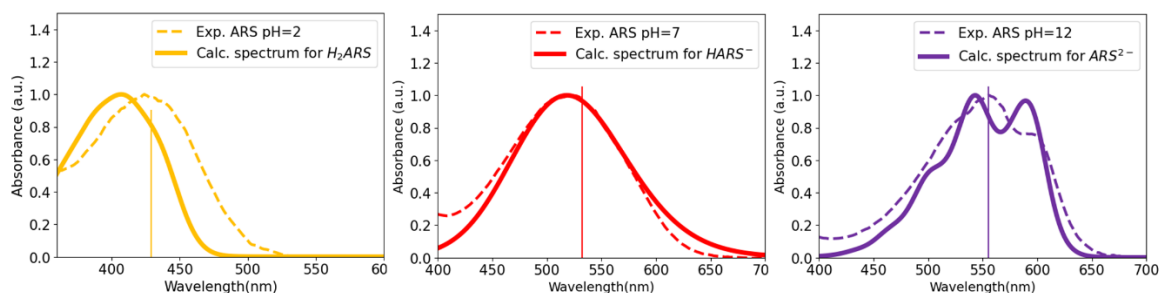


Fig. 6. Alizarin red S rotamer and deprotonated for each pH range, along with their relative energies. For the acidic form, the other forms are much higher in energy (>17 kcal/mol) and are not considered. The most stable forms are denoted H_2ARS (acidic form), $HARS^-$ (mono-deprotonated form), and ARS^{2-} (di-deprotonated form).

369

3.2.2. UV-Vis spectra prediction

370 The experimental spectra have maximum absorption energy at 425nm for H_2ARS , 521nm for $HARS^-$ and 560
 371 nm for ARS^{2-} with two shoulders at 600nm and 530nm. The computed spectra were done considering the
 372 method and protocol used for alizarin. The results are depicted in Fig.7. There is a better agreement
 373 between the experimental and computed spectra for the H_2ARS and $HARS^-$ form than alizarin, which can be
 374 explained by a higher solubility of alizarin red S. The computed VE matches perfectly the experimental one.
 375 Similarly, the AH spectra of the $HARS^-$ and ARS^{2-} are in adequation with the experimental ones, in both the
 376 topology and position of the spectra.









Form	Experimental (normalized)				Calculated (normalized)				ΔE_{2000}
	L*	a*	b*	color	L*	a*	b*	color	
H_2ARS	99.6	-10.8	77.3		99.2	-14.0	68.0		3.3
$HARS^-$	65.4	54.8	-3.2		59.1	57.7	-17.8		8.3
ARS^{2-}	54.4	51.0	-50.9		57.0	61.7	-53.6		4.0

Fig. 7. (Top) The calculated and experimental UV-Vis spectra for alizarin red S at different pH. The dotted line is the experimental one. The full lines are the calculated spectrum with Adiabatic Hessian (AH) method. The vertical lines correspond to the computed VE. (Bottom) Predicted and computed colors of different alizarin red S forms with CIELAB coordinates (L^* , a^* , b^*) and the calculated color variation between computed and experimental color coordinates with ΔE_{2000} .

377 To assess the quality of the color prediction, the calculated and experimental color CIELAB are represented
 378 in Fig.7. The calculated color matches the observed experimental color, with higher accuracy for the H_2ARS
 379 form (3.31 for alizarin red S and 13.32 for alizarin, see Fig.5). This can be explained again by its higher
 380 solubility compared to alizarin.
 381 The additional sulfonic group does not change the color of the compound compared to alizarin. This can be
 382 explained by the difference densities plotted in Fig.8. The sulfonic group does not participate in the electron
 383 delocalization for the $GS \rightarrow S_1$ transition.

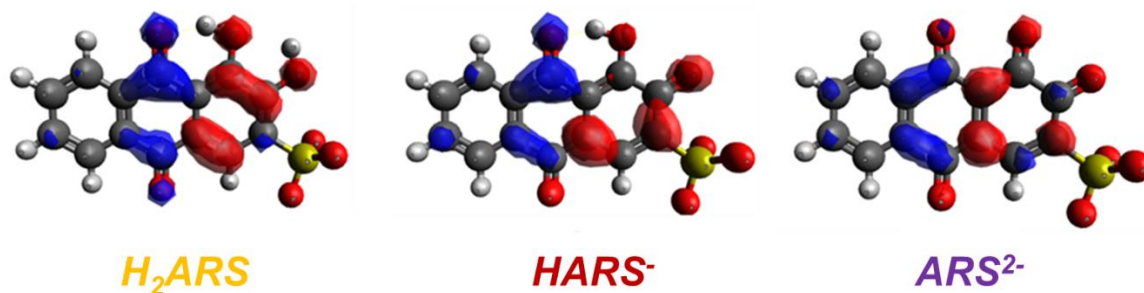


Fig. 8. Difference electronic densities for the $GS \rightarrow S_1$ transition for alizarin red S for different protonation states. The blue orbital represents a decrease in electronic density. The red one represents an increase in electronic density. The isovalue is 0.02 a.u.

384

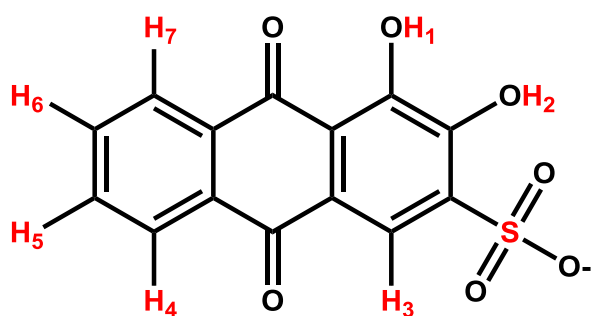
385 3.2.3. 1H and ^{13}C NMR prediction

386

387 Thanks to the higher solubility of alizarin red S in water, the measure of NMR spectra in D_2O is possible. We
 388 have obtained the 1H NMR spectra of H_2ARS in acetone- D_6 only as the one in water gives very broadened
 389 signals, probably due to exchange processes at room temperature which is consistent with other
 390 publications [26]. Following the best computational protocol for alizarin, the calculations were done using an
 391 explicit solvation scheme. All the geometries are accessible in the supplementary data 14. The comparison
 392 between the computed 1H chemical shifts is represented in Table 2 (in acetone- D_6) and ^{13}C in Table 3 (for

393 both acetone-D₆ and D₂O). The error deviation of aromatic hydrogens (H3 to H7) is less than 0.2 ppm in
 394 average (which represents 2.1% in mean error deviation). This error is less than for alizarin (which has 2.8%
 395 mean error deviation). There is a better agreement with experimental data for alizarin red S, again explained
 396 by its higher solubility compared to alizarin. The labile hydrogens are less accurate with 17% of error
 397 deviation for H2 and 11% for H2. The experimental and computed shifts can be found for different alizarin
 398 red S forms in supplementary data 8 to 13.

Table 2. Computed and experimental chemical shift of alizarin red S in acetone-D₆ with the respective error deviation in ppm or %.

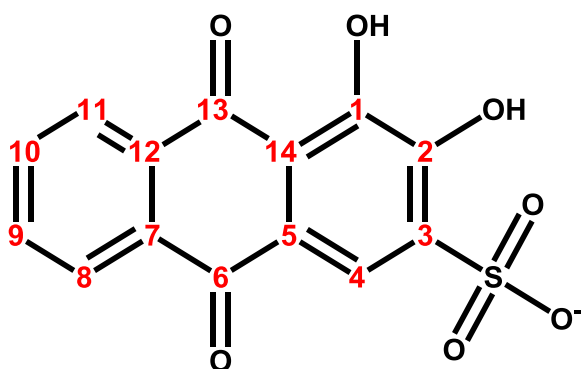


Attribution	Exp. (ppm) in acetone-D ₆	Calc. (ppm)	Calc.-error (ppm)	Error deviation (%)
H1	12.8	15.0	2.2	17.1
H2	11.9	13.2	1.3	11.1
H3	8.2	8.4	0.2	2.7
H4	8.3	8.5	0.2	2.7
H6-H5	7.9	8.0	0.1	1.3
H6-H5	7.9	8.0	0.1	1.3
H7	8.3	8.5	0.2	2.5

399
 400 The comparison between the computed and experimental ¹³C shifts in D₂O for alizarin red S is represented in
 401 Table 3. It shows that the error deviation of Carbons C4-C14 are less than 6 ppm on average (which
 402 represents 4.4 % in mean error deviation). There is a higher error deviation for carbons that are next to a
 403 hydroxy or sulfonic group (C1-C3) with up to 5.5% error deviation.
 404 Overall, there is a good agreement between the computed and experimental chemical shifts for both ¹H and
 405 ¹³C NMR spectra. This protocol proves to be efficient in predicting NMR spectra.
 406

Table 3. Computed and experimental chemical ^{13}C shift of alizarin red S in acetone- D_6 or D_2O with the respective error deviation in ppm or %.

Attribution	Acetone- D_6		D_2O	
	Calc.-error (ppm)	Error deviation (%)	Calc.-error (ppm)	Error deviation (%)
1	8.2	5.5	9.8	6.5
2	7.9	5.2	8.6	5.7
3	6.7	5.0	4.7	3.5
4	5.0	4.2	3.9	3.3
5	4.0	3.3	5.5	4.5
6	6.9	3.8	6.7	3.7
7	4.2	3.1	6.5	4.9
8	4.4	3.4	4.7	3.7
9	3.5	2.5	5.2	3.9
10	3.4	2.5	6.2	4.7
11	3.9	3.1	4.4	3.5
12	4.4	3.3	6.7	5.1
13	5.3	2.8	7.0	3.7
14	4.3	3.7	4.8	4.1



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4. CONCLUSION

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Deciphering experimental spectra of natural dyes is a persistent challenge due to the presence of impurities, extraction difficulties, solubility issues, fast degradation, and so on. In this context, computational spectroscopy appears as a solution for the elucidation of complex structures of pigments. Here, we focus on Madder, a natural dye with historical importance. By combining computational insights with experimental data, our study has demonstrated the great efficiency of the optimized computational protocol for accurately predicting UV-Vis spectra, CIELAB color coordinates, and NMR shift of alizarin, the main coloring molecule of Madder, and its analog alizarin red S. In particular, we have found that the incorporation of conformer, deprotonated forms for each pH, solvation models, and careful consideration of solvent interactions resulted in accurate description when compared with experimental measurements. The

418 computed spectra closely match the experimental data, providing a reliable foundation for the prediction of
419 other hydroxyanthraquinones and numerous colored dyes. The computation of CIELAB coordinates of these
420 2 molecules also yields good results despite some issues due to solubility. This approach can be of interest to
421 extracting the contribution of each dye in a mixture or for predicting new hues of colored molecules. By
422 overcoming experimental complexities, this protocol accelerates the development of compounds tailored to
423 specific needs by predicting with accuracy several properties. This optimized computational protocol
424 outlined here offers a versatile tool for designing novel molecules across a wide variety of coloring
425 industries, such as textiles, cosmetics, food, pharmaceuticals, and art. Furthermore, it can set the stage for
426 deeper exploration into more complex structures of pigments, which are often challenging to elucidate by
427 experimental methods.

428

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