Question 1. Solvent Effect on UV/Vis Spectra. (20 points)


Figure 2. Linear correlation between the $E_T(30)$ values of betaine dye 36 (cf. Table 2) and the $Z$ values of probe $53^{61,207,210}$ (cf. Table 1), measured in 56 solvents of different polarity at 25 °C. Correlation equation: $Z = 13.49 + 1.26E_T(30)$ (pairs of values $n = 56$; correlation coefficient $r = 0.980$; standard deviation of the estimate $\sigma = 1.72$). For the sake of clarity only ten points are assigned to the corresponding solvent.
(a) Reichardt’s betaine 36 (shown on the bottom right in “Figure 2”) shows ____________ (positive, negative) solvatochromism, that is, the more polar the solvent, the larger is the ____________ (bathochromic, hypsochromic) shift of the absorption. Reichardt’s salt 53 (shown on the top left in “Figure 2”) shows ____________ (positive, negative) solvatochromism. (6 points)

(b) Briefly explain the solvatochromism of Reichardt’s betaine 36 (8 points)

(c) Briefly explain the solvatochromism of Reichardt’s salt 53 (6 points)
Question 2. Combination Problem: C₈H₆O₄. (20 points)

Given the formula and the IR-, MS- and H-NMR spectra

(a) determine the structure of the unknown and draw it on the right (6 p.),

(b) assign two significant peaks in the IR spectrum (4 p.),

(c) draw the M⁺ ion and its fragmentation to ion $m/z = 121$ directly in the MS spectrum (6 p.), and

(d) explain on the NMR spectrum why the peak above 13 ppm is broad. (4 p.)

From the Introduction: “Matrix-assisted laser desorption/ionization time-of-flight mass spectrum (MALDI-TOF-MS) is a very useful method to characterize macromolecules especially for the proteins (Nobel Prize in Chemistry 2002). Compounds 1 and 2 … are the most frequently used matrices for the desorption/ionization of macromolecules because they all have conjugated π-systems which can absorb the energy of the laser greatly and help to ionize the samples. 3,5-Dimethoxy-4-hydroxycinnamic acid, one of the derivatives of cinnamic acid (a.k.a. sinapinic acid), is also a well-known organic compound because it is proved to be one of the toxic chemicals in rapeseed. …”

Complete Structure of Sinapinic Acid (2 p.)


UV ABSORPTION OF MALDI MATRICES

<table>
<thead>
<tr>
<th>Matrix</th>
<th>337 nm $\alpha_{max}$ (cm$^{-1}$)</th>
<th>337 nm $\sigma_{max}$ (cm$^2$)</th>
<th>FWHM$_{max}$ (nm)</th>
<th>350 nm $\alpha_{max}$ (cm$^{-1}$)</th>
<th>350 nm $\sigma_{max}$ (cm$^2$)</th>
<th>FWHM$_{max}$ (nm)</th>
<th>OS$<em>{max}$/OS$</em>{min}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coumarin 120</td>
<td>9.69 x 10$^4$</td>
<td>3.91 x 10$^{-17}$</td>
<td>110</td>
<td>7.16 x 10$^{-17}$</td>
<td>54</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>Ferulic Acid</td>
<td>1.16 x 10$^5$</td>
<td>3.46 x 10$^{-17}$</td>
<td>81</td>
<td>6.10 x 10$^{-17}$</td>
<td>70</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Sinapinic Acid</td>
<td>1.10 x 10$^5$</td>
<td>4.34 x 10$^{-17}$</td>
<td>104</td>
<td>6.85 x 10$^{-17}$</td>
<td>60</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>α-CHC</td>
<td>2.18 x 10$^6$</td>
<td>1.04 x 10$^{-16}$</td>
<td>89</td>
<td>8.24 x 10$^{-17}$</td>
<td>59</td>
<td>1.90</td>
<td></td>
</tr>
<tr>
<td>2,5-DHB</td>
<td>7.95 x 10$^4$</td>
<td>1.98 x 10$^{-17}$</td>
<td>118</td>
<td>1.46 x 10$^{-17}$</td>
<td>44</td>
<td>3.64</td>
<td></td>
</tr>
<tr>
<td>Nicotinic Acid$^a$</td>
<td>$\sigma_{max}$ = 7.35 x 10$^4$</td>
<td>$\sigma_{max}$ = 7.35 x 10$^4$</td>
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<td>$\sigma_{max}$ = 7.35 x 10$^4$</td>
</tr>
</tbody>
</table>

$^a$ Note that $\sigma_{max}$ at 248 nm and 266 nm is given for nicotinic acid instead of at 337 nm as the absorption is very weak at this wavelength.
As can be seen from Table 1, sinapinic acid is used in conjunction with a 377 nm laser. We can be sure that sinapinic acid absorbs at that frequency; it would not be used otherwise. In fact, the absorption maximum in the UV/Vis spectrum of sinapinic acid is very close to 377 nm and we are getting essentially the greatest possible absorption. But what makes the absorption of sinapinic acid to occur almost in the visible?

Using your books and spectral data collections (e.g. Pretsch et al.), provide the following reference data (12 p.):

<table>
<thead>
<tr>
<th>What is the approximate wave length of the $\pi \rightarrow \pi^*$ excitation of <strong>acrolein</strong>?</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the approximate wave length of the $\pi \rightarrow \pi^*$ excitation of <strong>acrylic acid</strong>?</td>
</tr>
<tr>
<td>What is the approximate wave length of the $\pi \rightarrow \pi^*$ excitation of <strong>phenol</strong>?</td>
</tr>
<tr>
<td>What is the approximate wave length of the $\pi \rightarrow \pi^*$ excitation of <strong>anisol</strong>?</td>
</tr>
</tbody>
</table>

Considering the above data, argue why the absorption maximum of sinapinic acid occurs red-shifted (6 points).
Question 4. Mass Spectrometry of Milk. (20 points)


Isopropylthioxanthone (ITX) is used as photoinitiator in UV inks applied to paper- or plastic-based packaging materials. ITX has recently been found in a number of foods such as ready-to-feed infant formula.

![Chemical structure of isopropyl-9H-thioxanthen-9-one](image)

The HPTLC-ESI/MS spectrum is shown (at 1 μg on silica gel plates). The experimental section contains the following two passages:

“Silica gel 60 (article no. 1.05641) HPTLC plates and RP18 HPTLC plates (article no. 1.05914), both 20°—10 cm (Merck, Darmstadt, Germany) were employed as stationary phases.”

“When acidic samples like milk or yoghurt were neutralized with sodium hydrogen carbonate (addition of 100 mg to 4 g sample).”

The authors claim that two peaks in the spectrum demonstrate the presence of ITX.

<table>
<thead>
<tr>
<th>Molecular Formula of ITX itself (4 p.)</th>
<th>Molecular Mass of ITX itself (4 p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak #1: m/e and structure (6 p.)</td>
<td>Peak #2: m/e and structure (6 p.)</td>
</tr>
</tbody>
</table>
Question 5. Mass Spectrometry of Plum Extracts. (20 points)


![Graph showing mass spectrometry data of plum extracts.](image)

**Figure 3.** ESI LC/MS/MS of six chlorogenic acid isomers identified in dried plum extract.

**ESI MS/MS with negative ion mode:**

![Chemical structure of chlorogenic acid and cryptochlorogenic acid](image)

cryptochlorogenic acid

\[ [\text{M-H}] 353 \]
Abstract of the paper. Dried plums are known as a healthy food in the West and used as medicine in India. They have been characterized by high concentrations of phenolic compounds, which are believed to play a crucial role in protection against various age-related diseases. Liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) with four different conditions was used to analyze the phytochemicals in commercial dried plums. The major components were neochlorogenic acid and cryptochlorogenic acid. Forty minor components were characterized by their MS/MS spectra and LC retention time. Six of them are novel ester isomers formed by two caffeic acids and one quinic acid. The diagnostic fragmentation patterns of different phenolics are presented on the basis of electrospray ionization (ESI) MS/MS data of components in dried plums and fourteen authentic standards.

Provide complete structures of the four fragment ions generated in the ESI-MS/MS of ion 353. A complete structure means that you show all atoms, any lone pairs, any radical sites, and any formal and actual charges.

\[
\begin{array}{ccc}
  m/z = 191 & & m/z = 179 \\
  m/z = 173 & & m/z = 135 \\
\end{array}
\]