

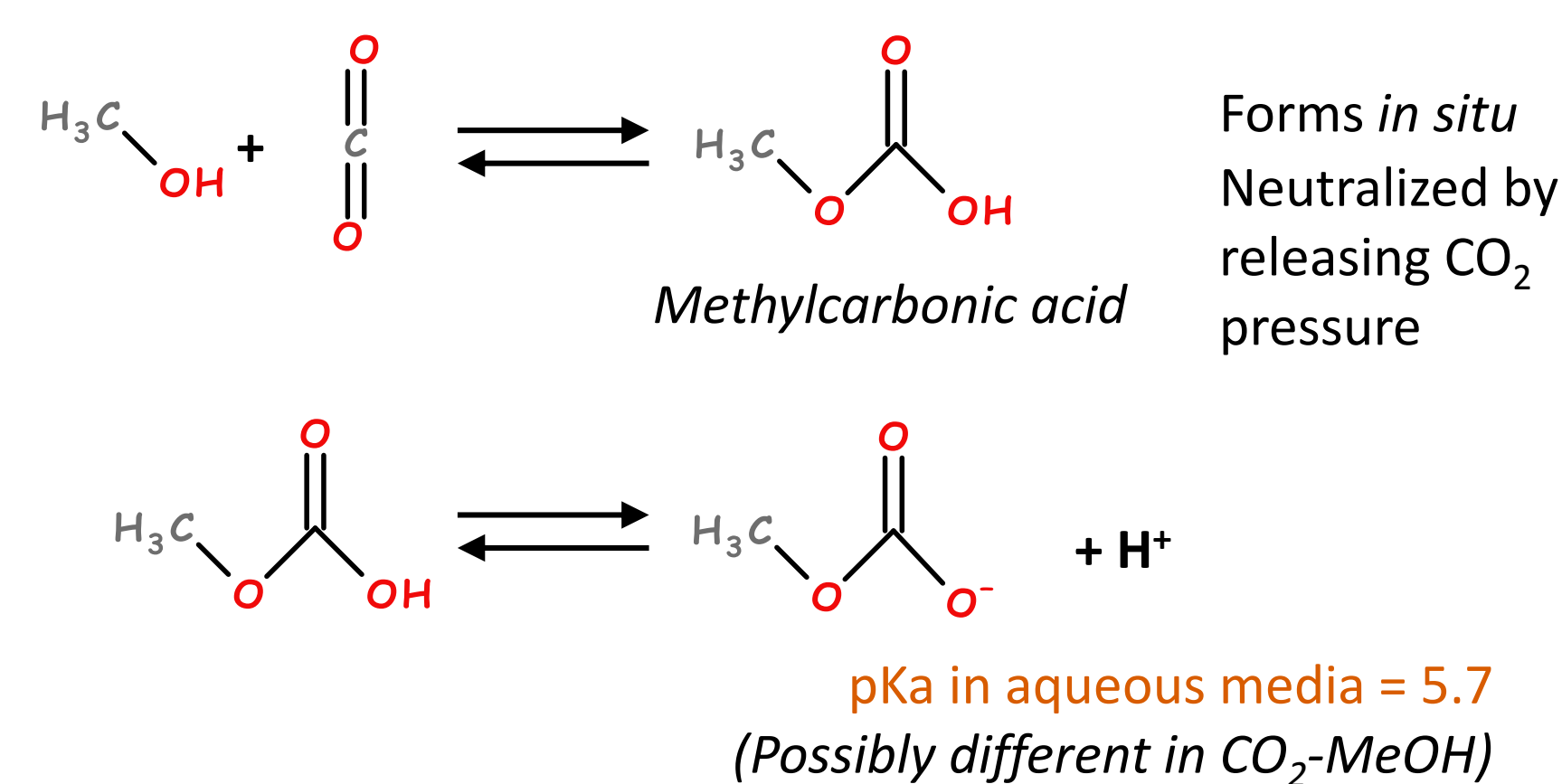
Introduction

Chromatographic retention of acidic and basic compounds in SFC is highly sensitive to the ionization state of the solute, as is the case in aqueous HPLC. Therefore, the apparent pH and apparent pK_a of analytes in the carbon dioxide - based mobile phases are important parameters to estimate for the optimization of SFC separations. However, due to the difficulties in measuring pH in a pressurized fluid, so far acid-base properties of carbon dioxide-solvent mixtures have been the subject of only rare studies¹. It is however known that carbon dioxide and alcohol react to form alkylcarbonic acid, thereby causing some acidity².

1. D. Wen, S.V. Olesik, Anal. Chem. 72(3), 2000, 475-480.

2. RR. Weikel et al., Ind. Enf. Chem. Res. 46, 2007, 5252-5257

Methanol acts as an acidic additive



Methodology

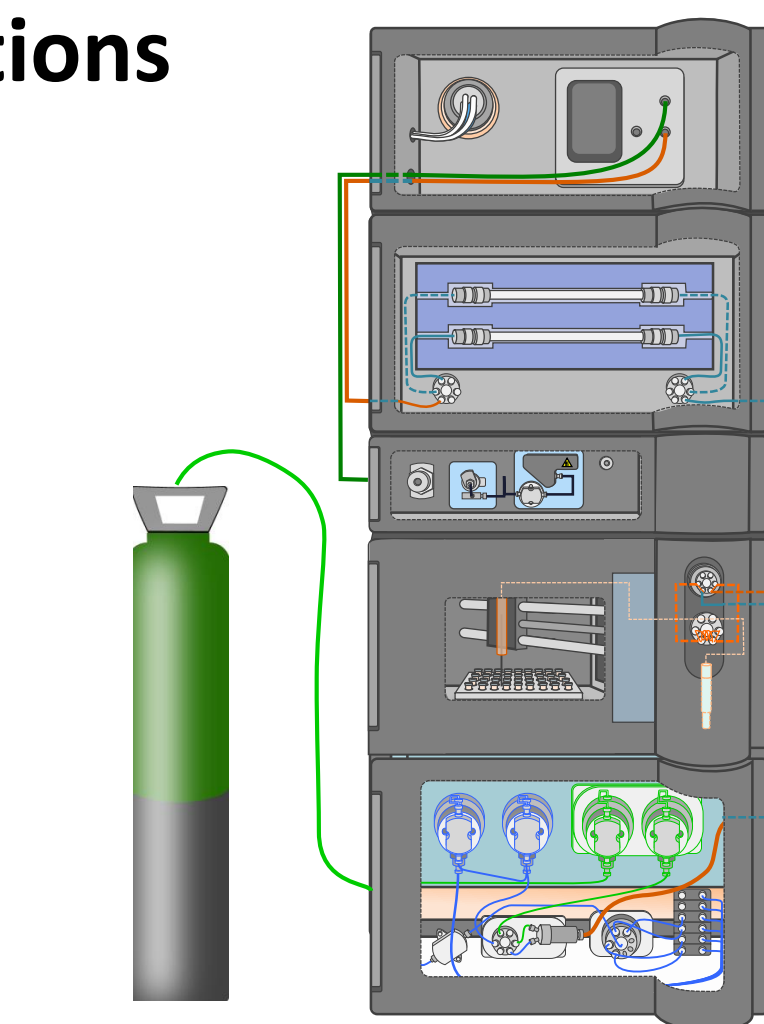
To try and determine the relative acidity of commonly used SFC mobile phases, the UV-visible spectra of five well-chosen pH indicators measured in carbon dioxide-methanol mixtures in varied proportions, with or without additives (acids, bases or salts, all 20 mM in methanol), were compared to the spectra obtained in liquid methanol, buffer and methanol-buffer mixtures. Principal component analysis (PCA) was used to compare all spectral data and provide an estimation of the acidity surrounding an analyte in SFC.

Operating conditions

Liquid composition:
Citrate-phosphate buffer
pH varying from 1 to 11
with or without methanol
in varied proportions (0 to 100%)



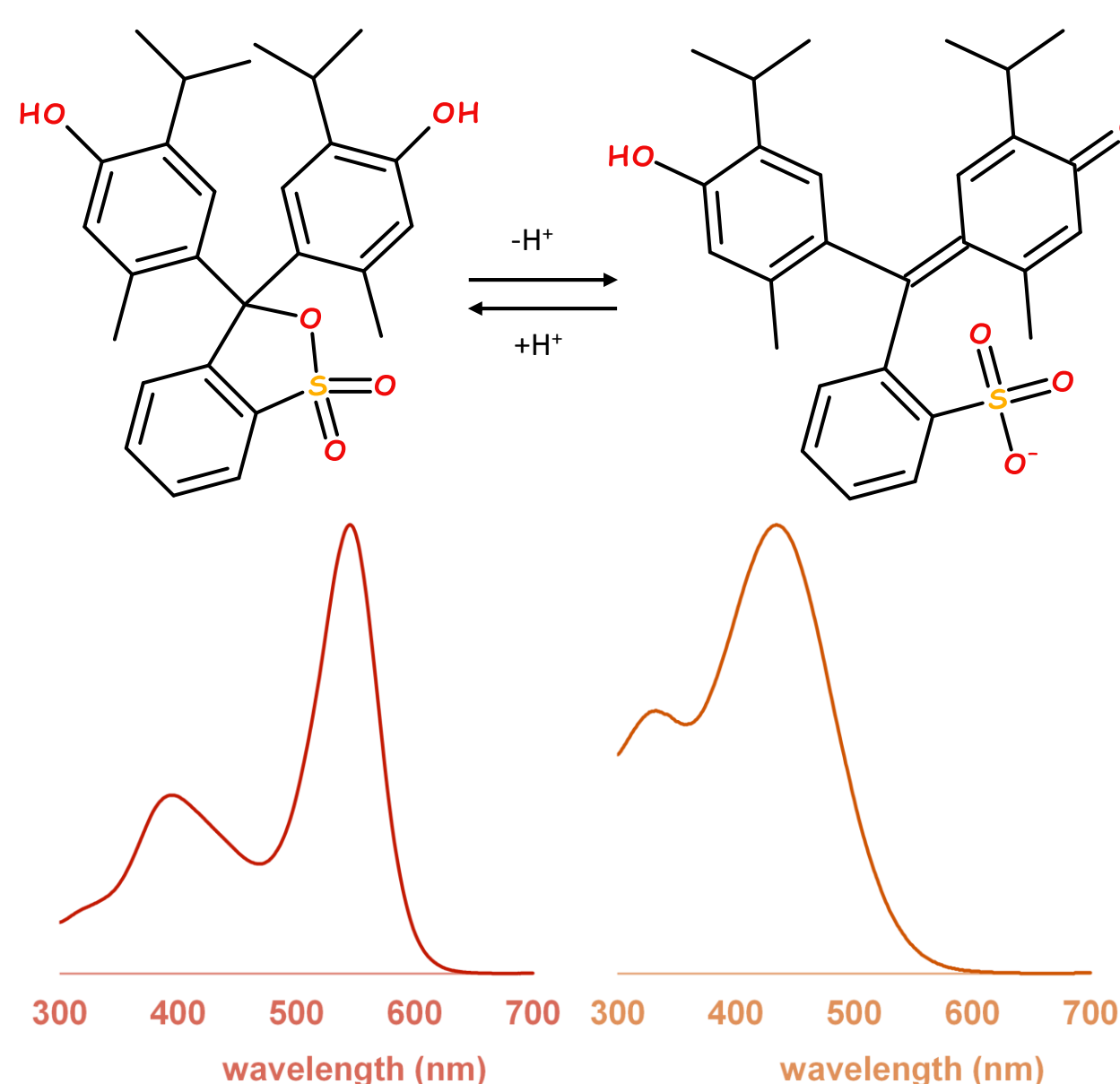
Shimadzu UV-1800



ACQUITY UPC²

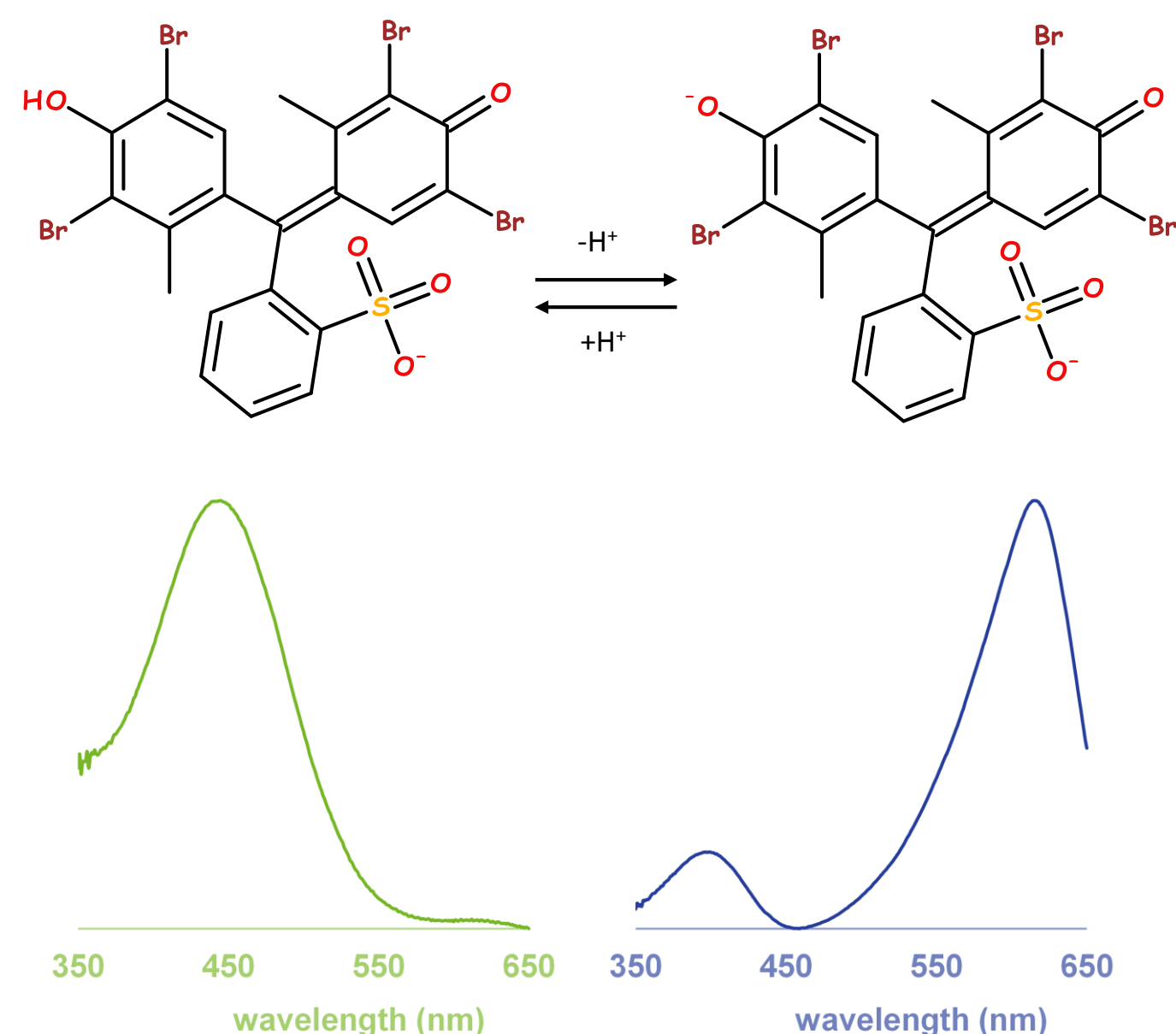
Detection:
Diode-array detector
300-700 nm
Column:
PEEK tubing
Conditions:
30°C, 15 MPa
Test-compounds:
5 pH indicators
(see details below)
Mobile phase:
CO₂-methanol
(with or without additives)
In varied proportions
3 mL/min

Thymol Blue



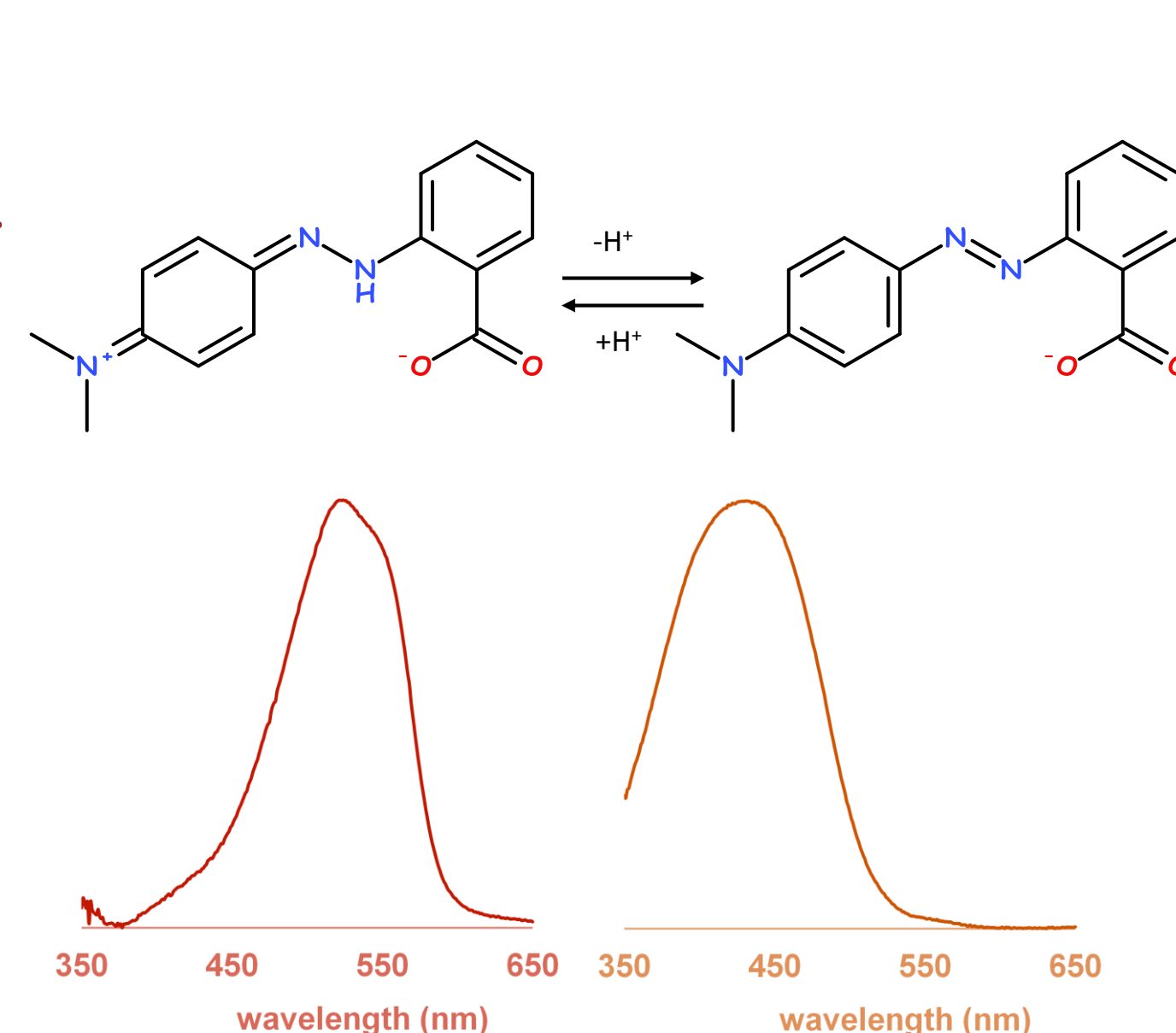
1.7

Bromocresol Green



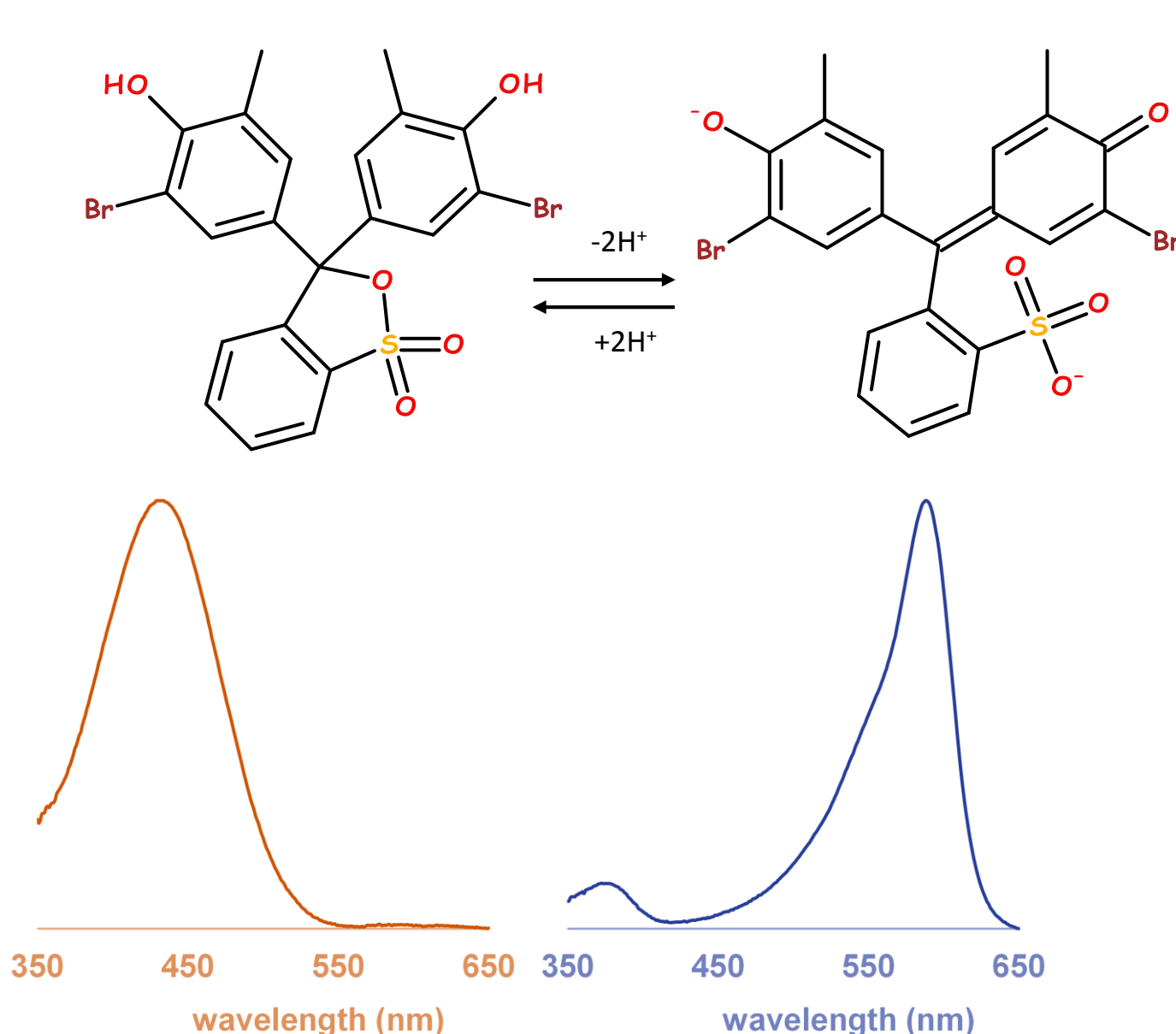
4.7

Methyl Red



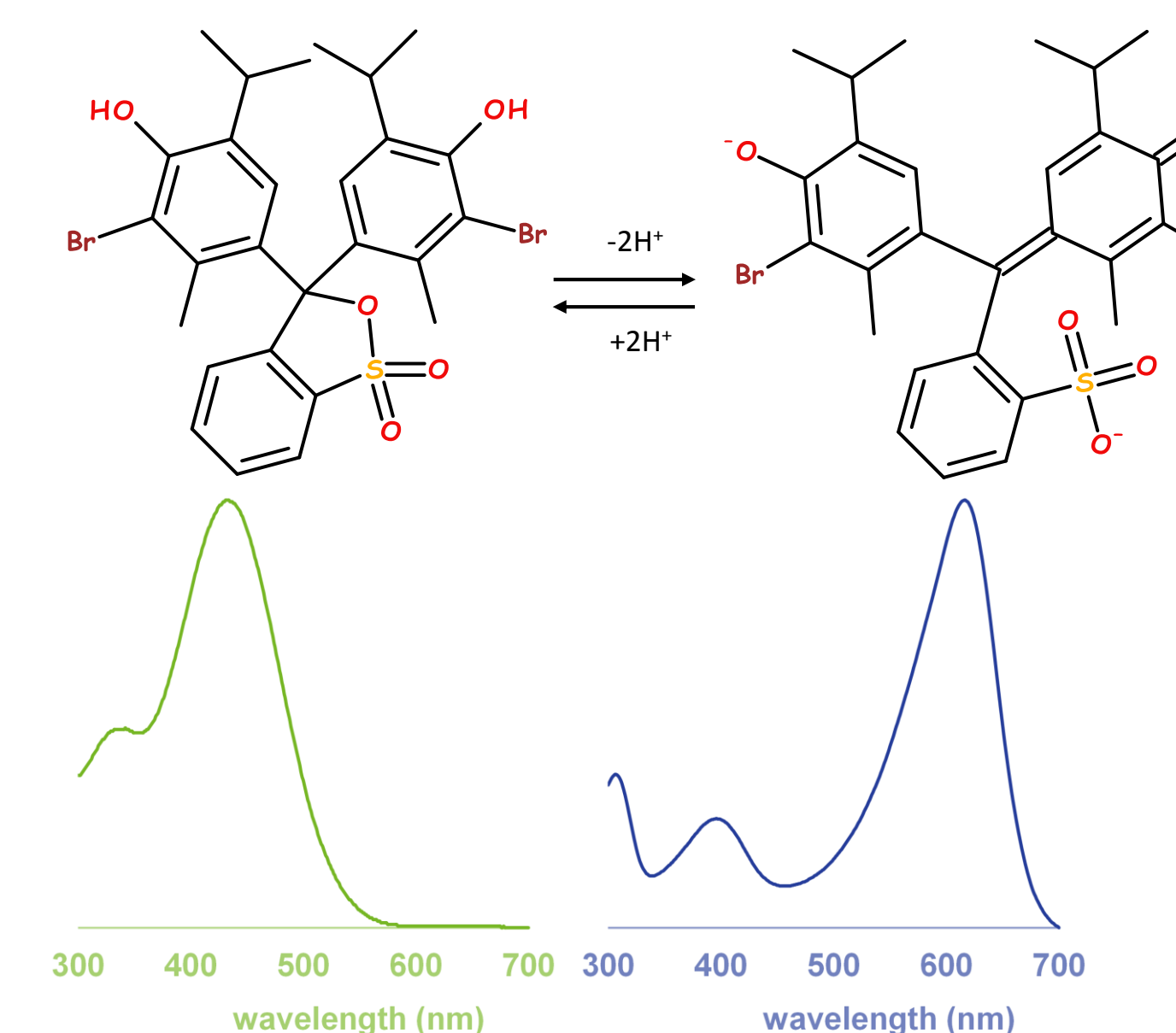
5.1

Bromocresol Purple



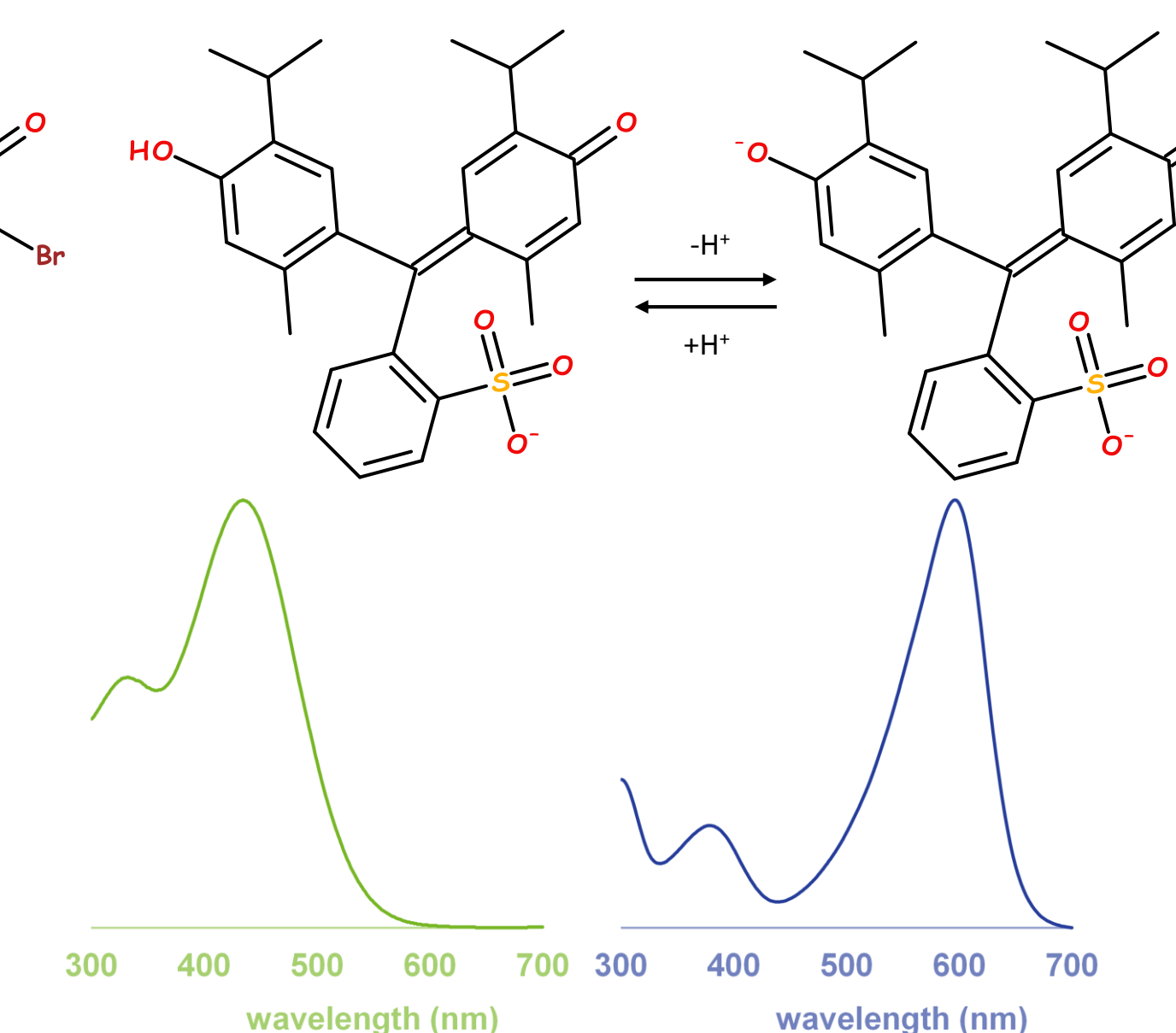
6.3

Bromothymol Blue

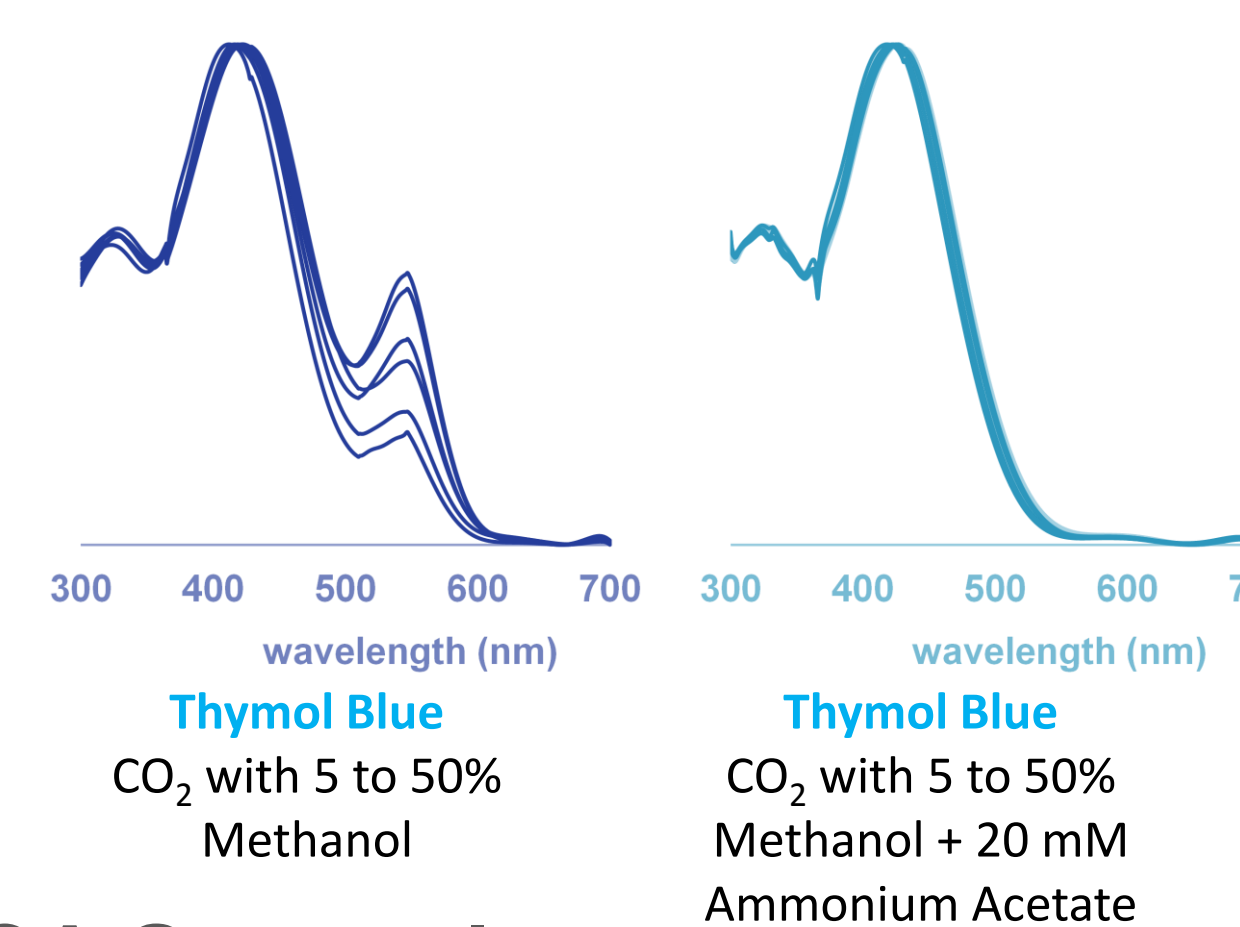
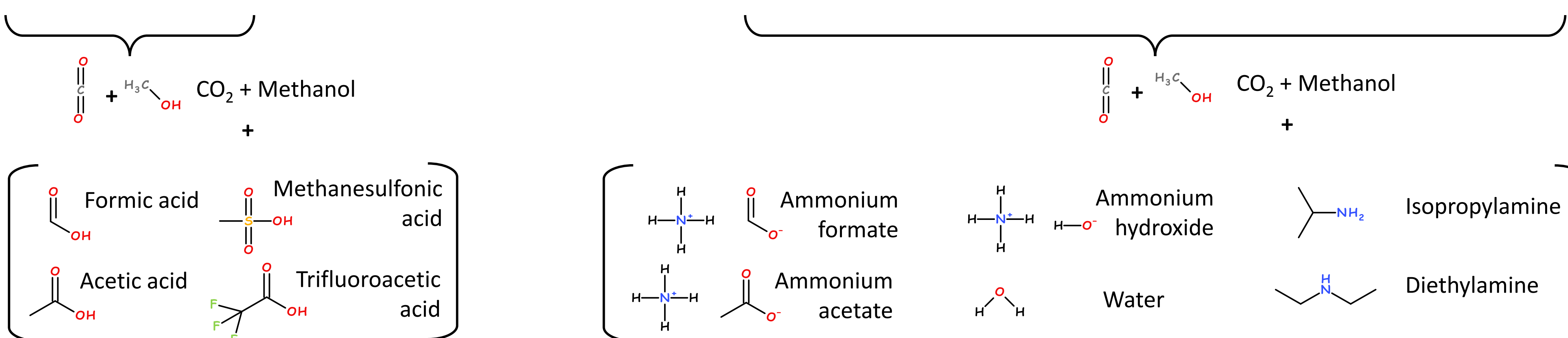


7.1

Thymol Blue



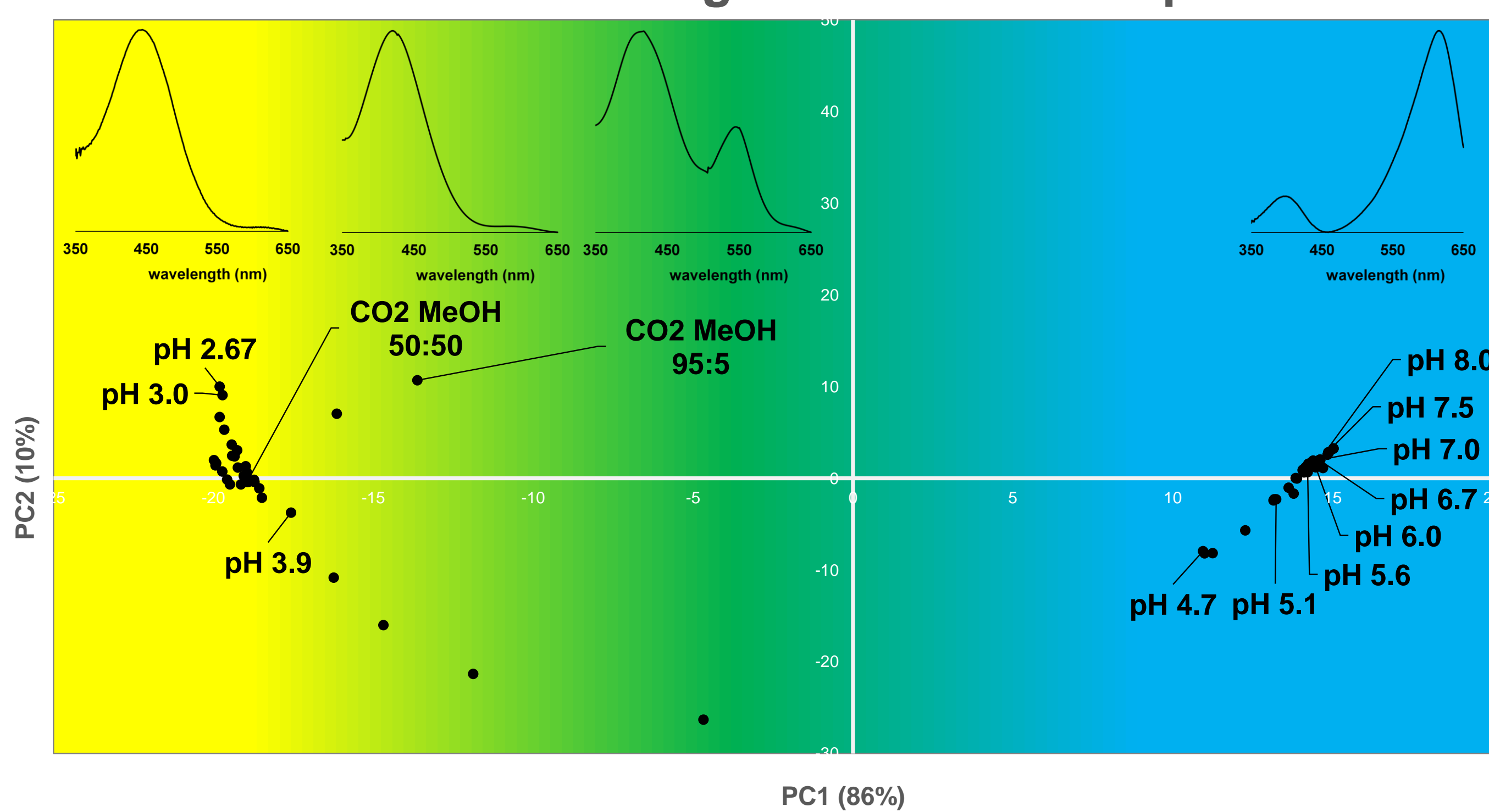
8.9



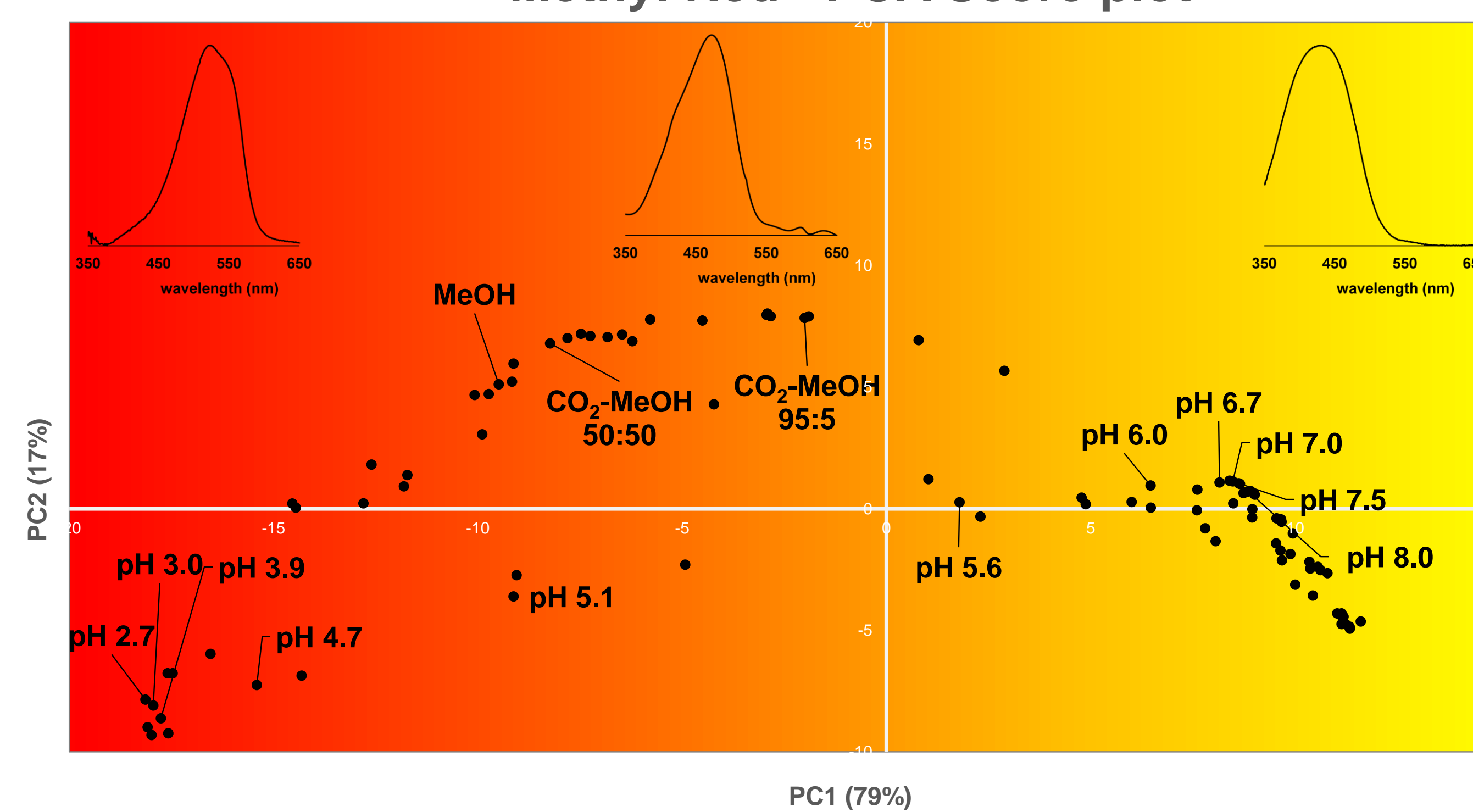
Effects of mobile phase additives

When methanol is employed alone (without any additive), the UV-visible spectra measured are seen to vary when methanol proportion changes. First, the position of absorption maxima is shifting somewhat, as could be expected from a more or less polar environment, but also the proportion of acidic and basic species, resulting from a change in the acidity of the mobile phase composition. More precisely, large proportions of methanol result in more acidic conditions. It can be seen on the figures on the left that the presence of a salt additive (ammonium acetate, 20 mM in methanol) in the mobile phase composition seems to have a stabilizing effect on the apparent acidity because the UV spectra show less bathochromic shift and no change in the proportions of acidic and basic species. It may then be inferred that the polarity and acidity in the cybotactic region will be more stable over a gradient elution when an additive is present.

Bromocresol green - PCA Score plot



Methyl Red - PCA Score plot



Conclusions

Based on the comparison of UV spectra recorded in SFC conditions and UV spectra recorded in aqueous buffers, the acidity of carbon dioxide – methanol mixtures is likely equivalent to aqueous acidity of pH 4-5. Increasing the methanol proportion (5 to 50%) is slightly shifting the apparent pH towards more acidic values. The use of acidic additives (formic acid, acetic acid, trifluoroacetic acid, methanesulfonic acid) is causing a further decrease of apparent pH, down to 1. The use of basic additives (diethylamine, isopropylamine), ammonium hydroxide or salts (ammonium acetate, ammonium formate) causes no significant change of the apparent pH, but is somewhat helpful in stabilizing a constant acidity over a methanol gradient.