



SimulationsPlus

Clofazimine pK_a Determination: the Underestimated Yet Significant Influence of Molecular Aggregation

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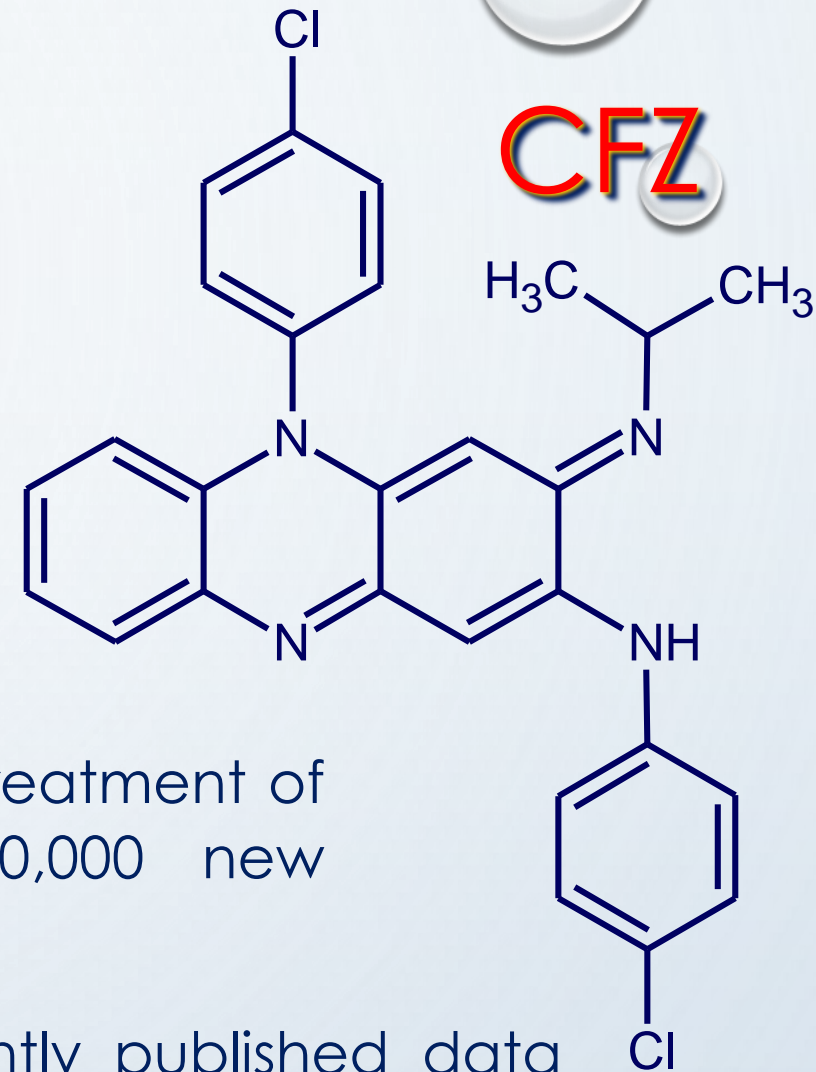
Clofazimine

Practically insoluble antibiotic and anti-inflammatory drug, first synthesized in 1957.

Active against *Mycobacterium leprae*, clinically used to treat leprosy (Hansen's disease).

Much interest in repurposing **clofazimine (CFZ)** for the treatment of tuberculosis; globally, there are approximately 500,000 new tubercular cases every year.

The interest in **CFZ** has further increased since recently published data show that it possesses **inhibitory activity against several coronaviruses** and can antagonize the replication of **SARS-CoV-2 and MERS-CoV** in a range of *in vitro* systems.



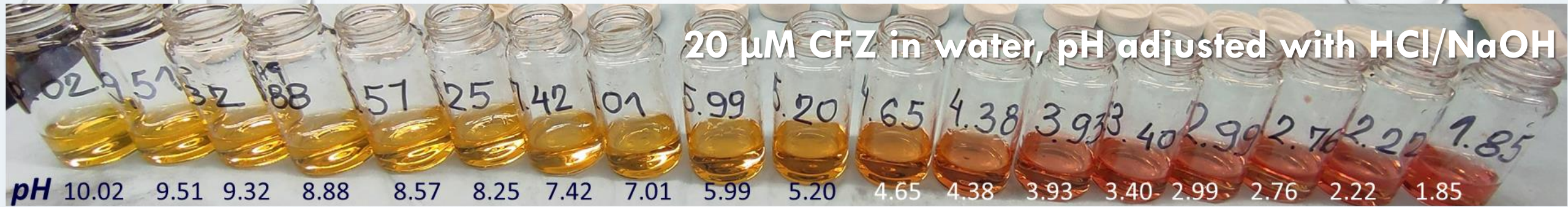
Although **CFZ** has been known since 1957 and used therapeutically since 1986, its **pK_a value was not confidently known**. Previously reported measured (Table 1) and predicted (Table 2) pK_a values span 3 orders of magnitude.

pK _a	SD	t (°C)	I (M)	Method
6.08	0.002	25	–	Solubility-pH ^a
8.35	0.09	–	–	–
8.37	–	–	–	Potentiometric
8.51	–	37	–	Spectrophotometric
9.11	–	25	0.025	CE/MS

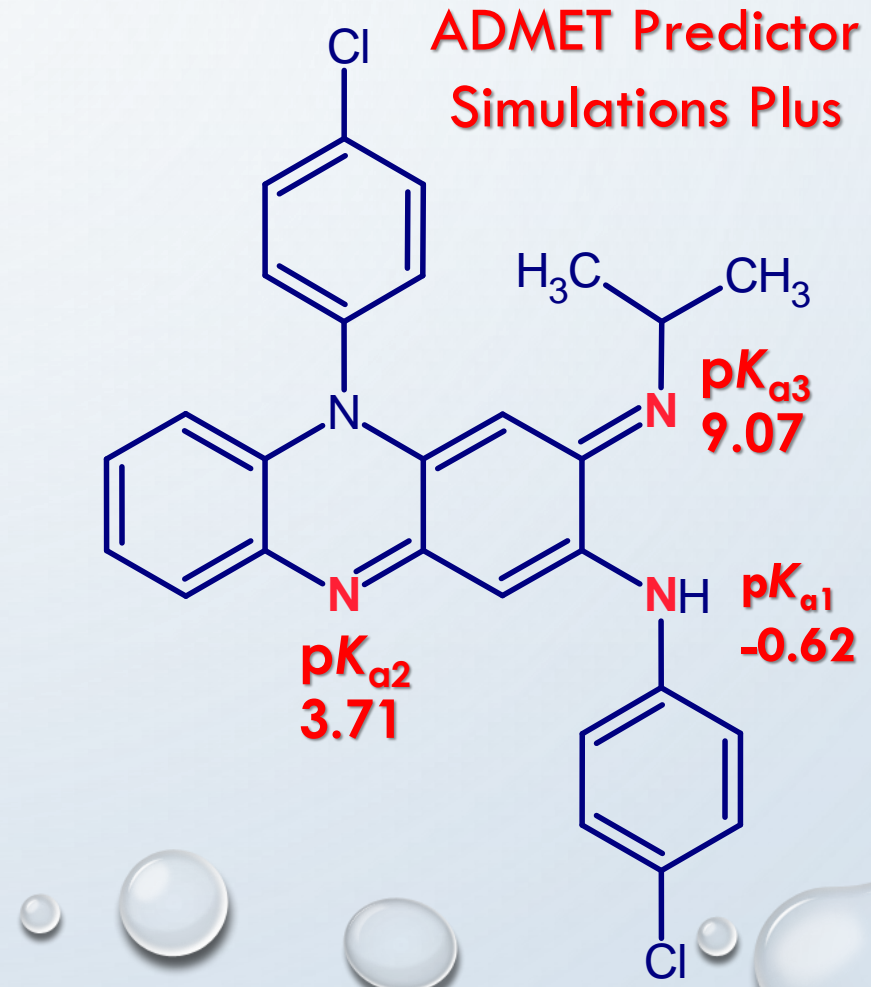
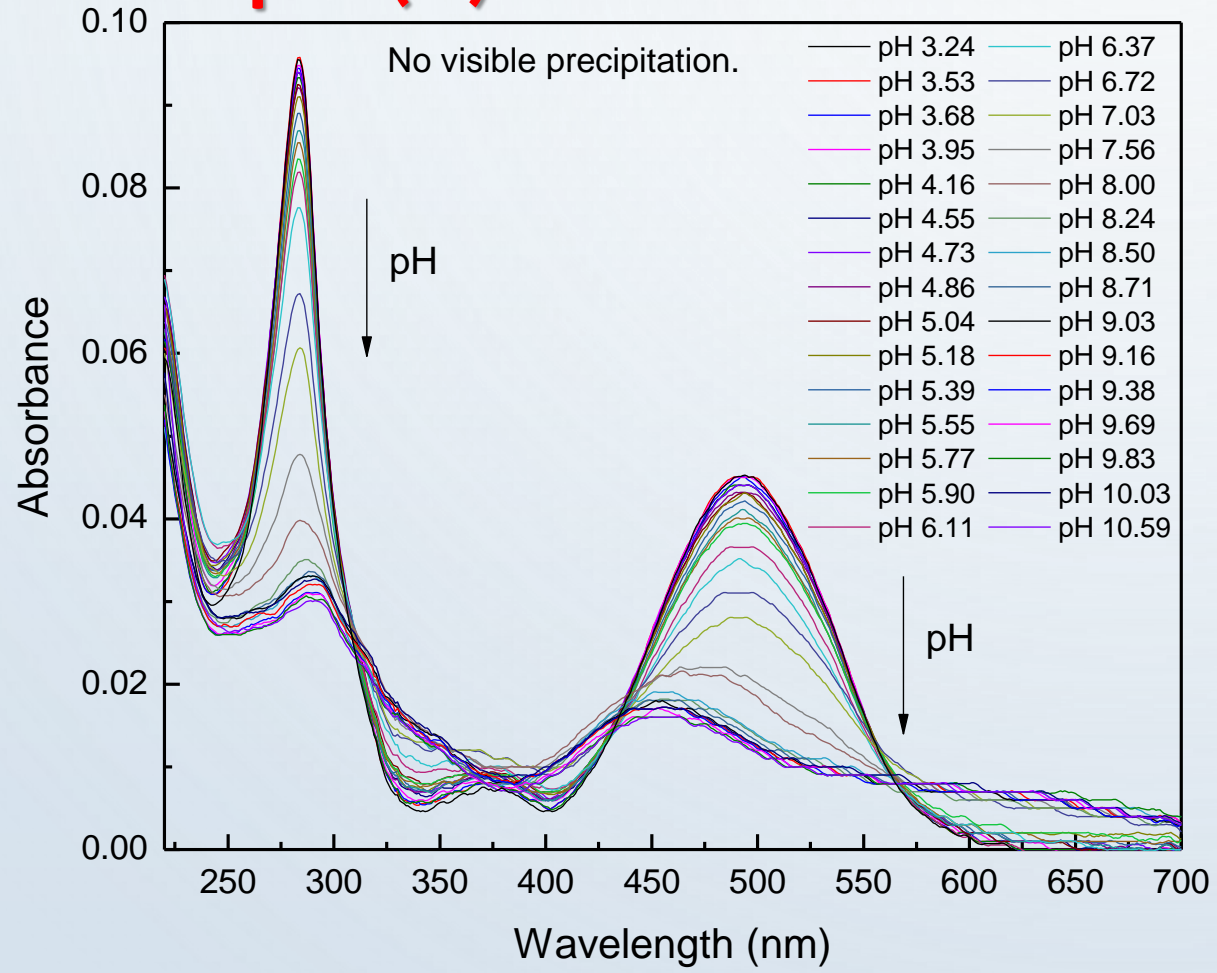
Table 1. Reported measured pK_a values of **CFZ**

Table 2. Calculated pK_a values of **CFZ**

pK _a	Software	Version
5.48	ChemAxon MarvinSketch	5.3.7
6.24	ACD/Labs	3.0
8.70	ADME Boxes (ACD/Labs)	4.9
9.29	ChemAxon MarvinSketch	5.12.0
9.07	ADMET Predictor	10.4



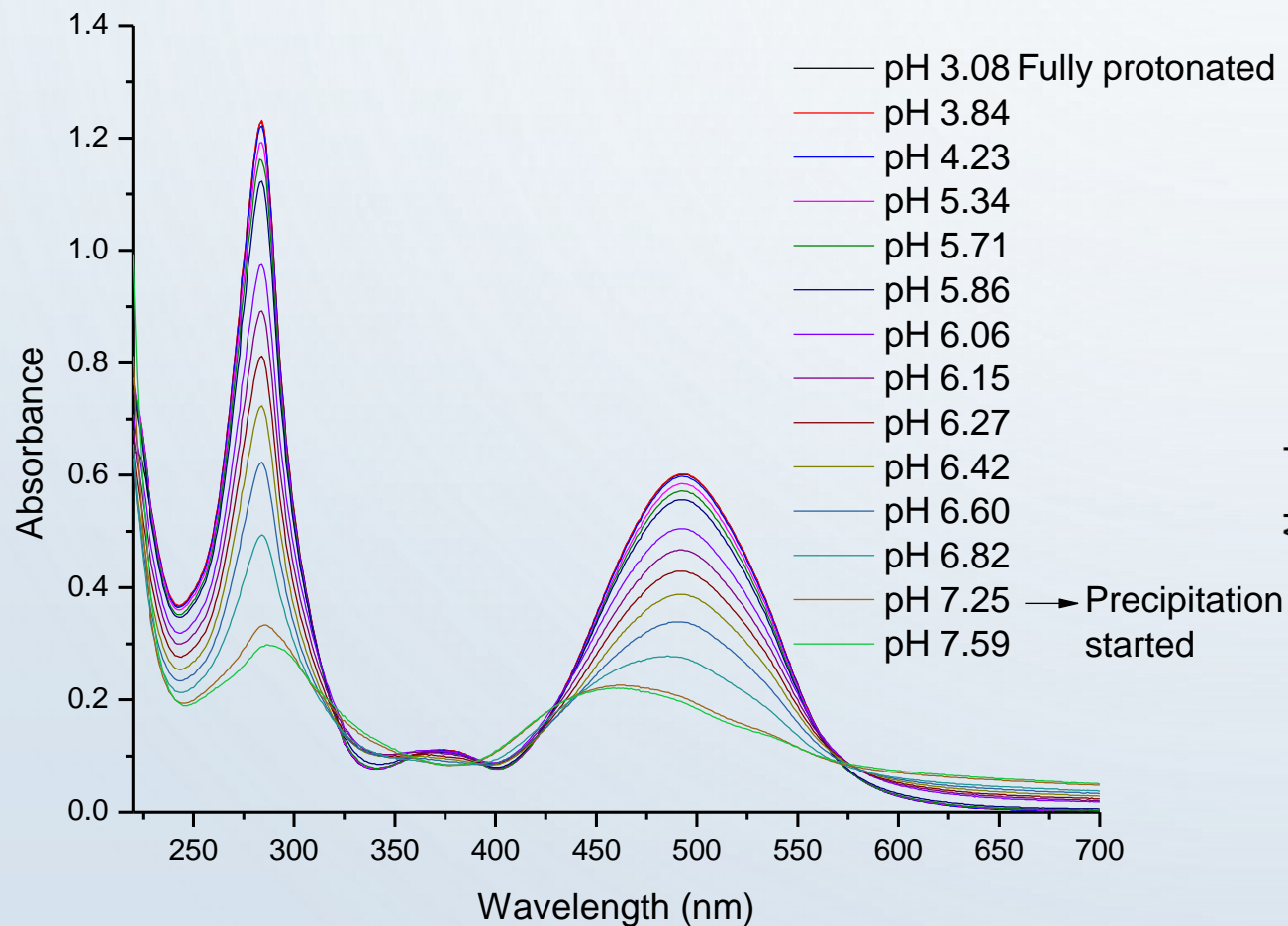
2 μM (!!!) CFZ in 10 mM PBS



20 μM CFZ in 10 mM HEPES

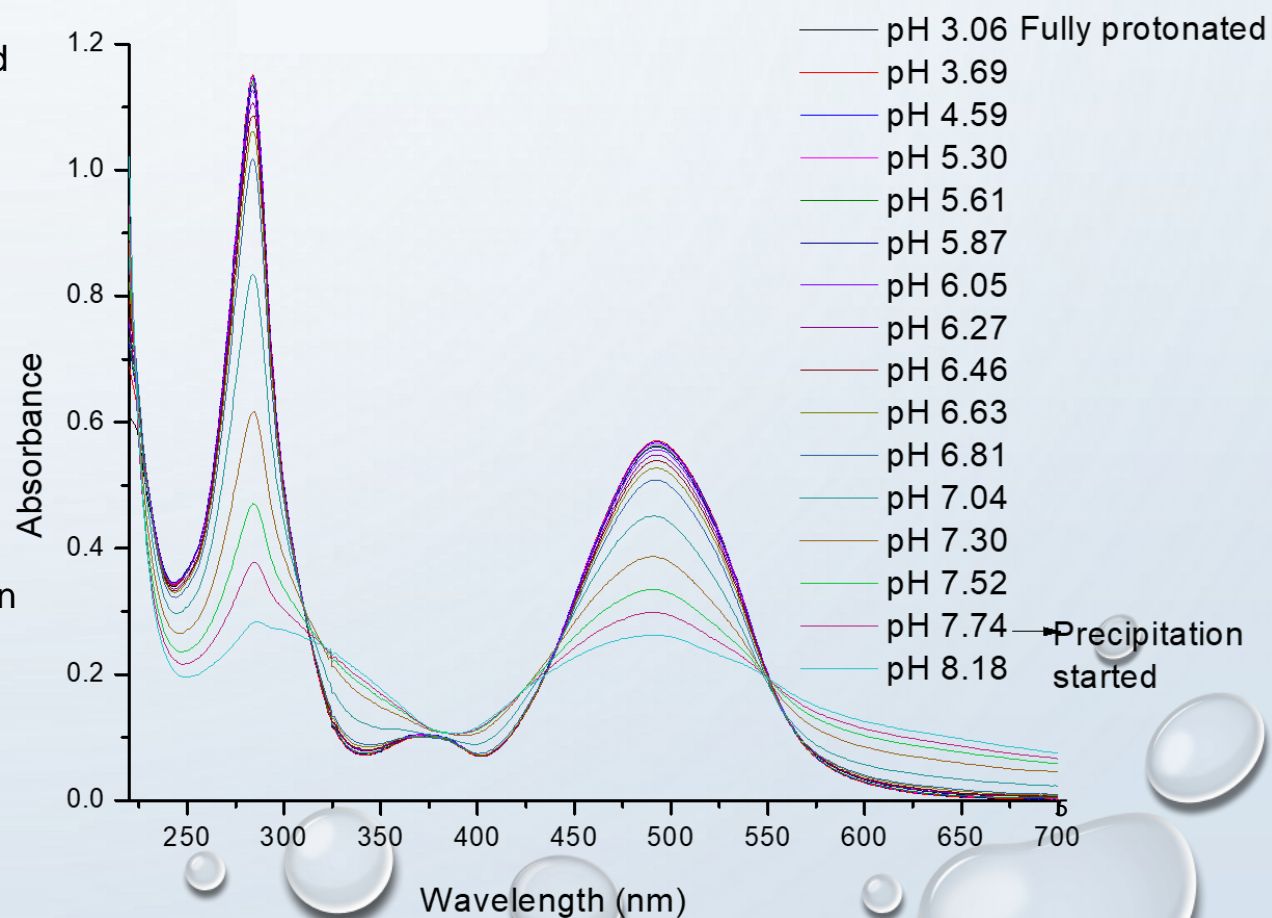
Pure aqueous media

Albert and Serjeant: $\text{p}K_a \sim 6.5$



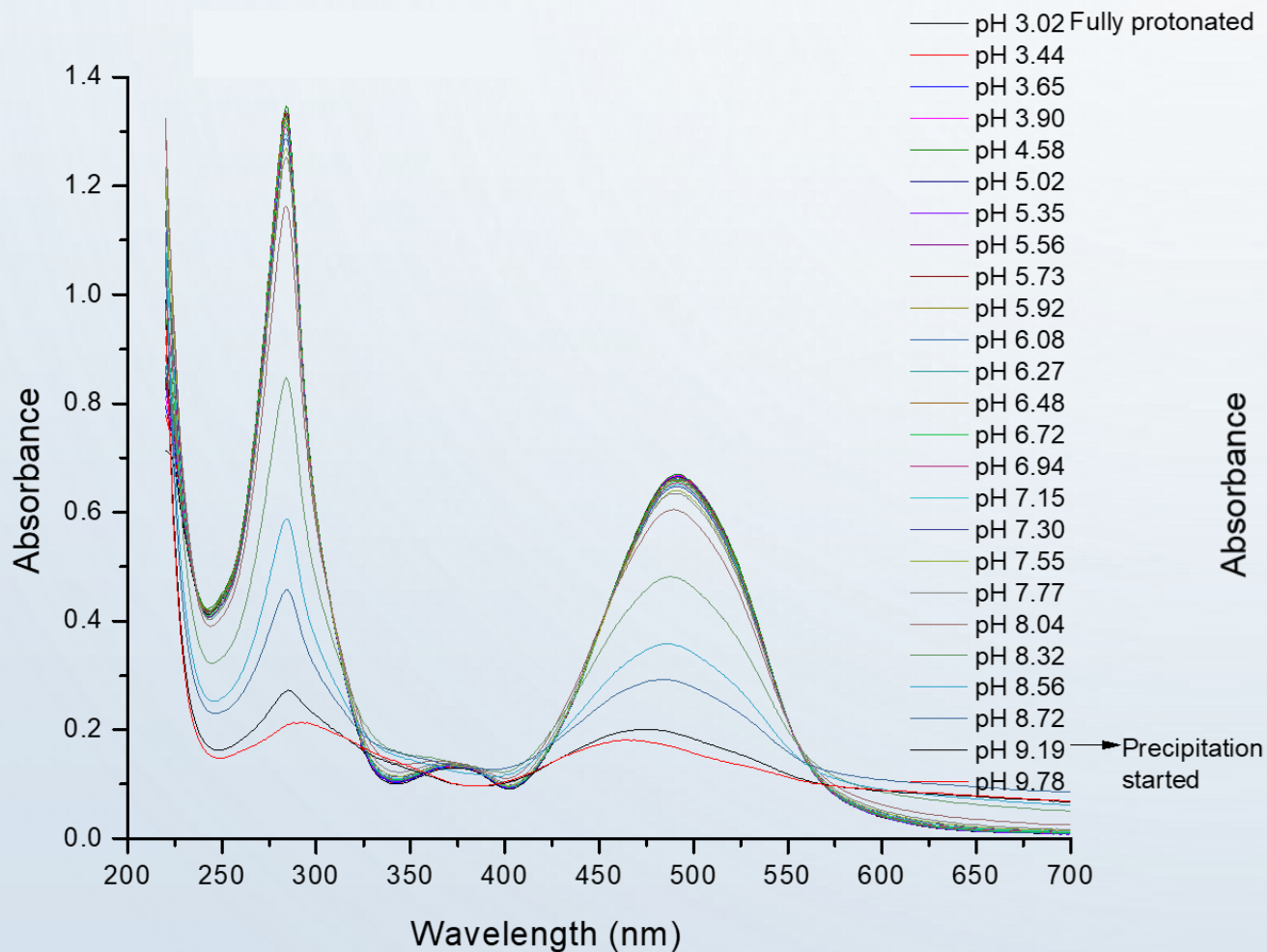
20% (volume) MeOH

Albert and Serjeant: $\text{p}K_a \sim 7.2$



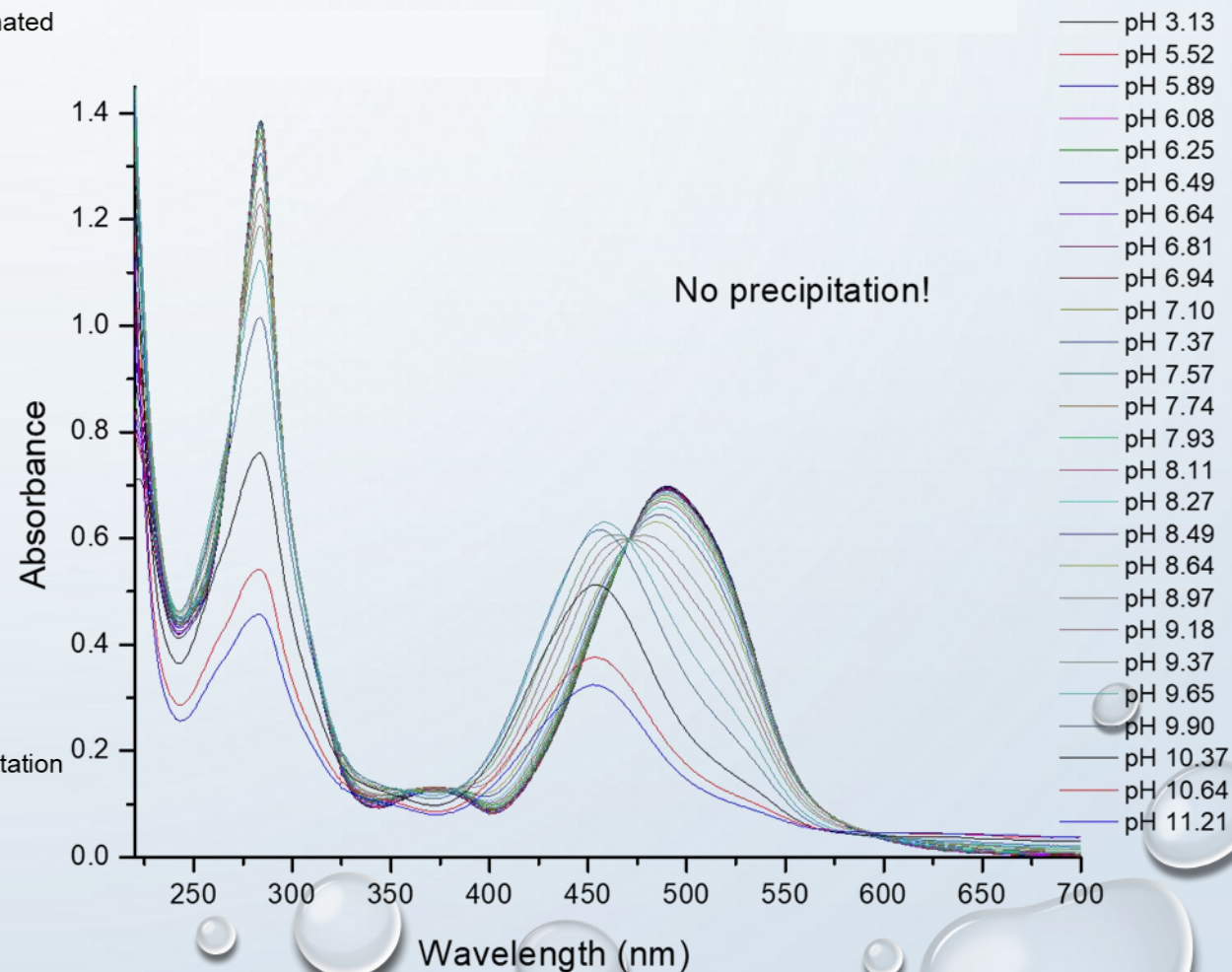
40% (volume) MeOH

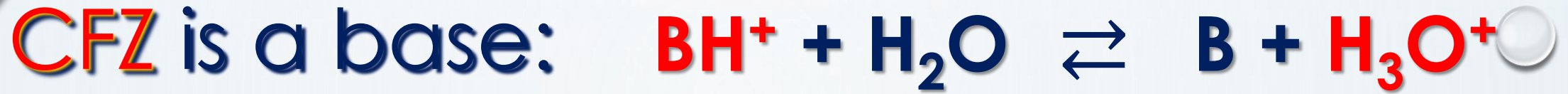
Albert and Serjeant: $pK_a \sim 8.5$



60% (volume) MeOH

Albert and Serjeant: $pK_a \sim 9.1$

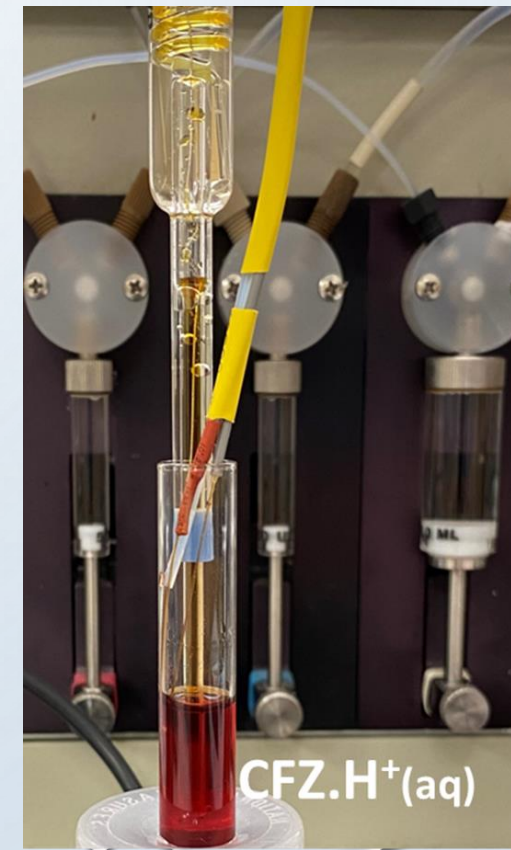
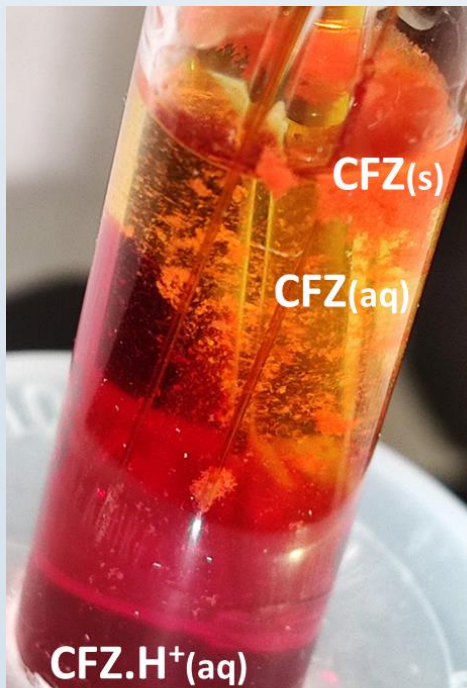




$\text{p}K_a$ Value of a base should decrease (!!!) as dielectric constant decreases (% MeOH increases).

So, what did we see in spectra?
(except nice color change)

Potentiometric titrations (12):
0.2-0.9 mM CFZ in 46-75 wt% MeOH

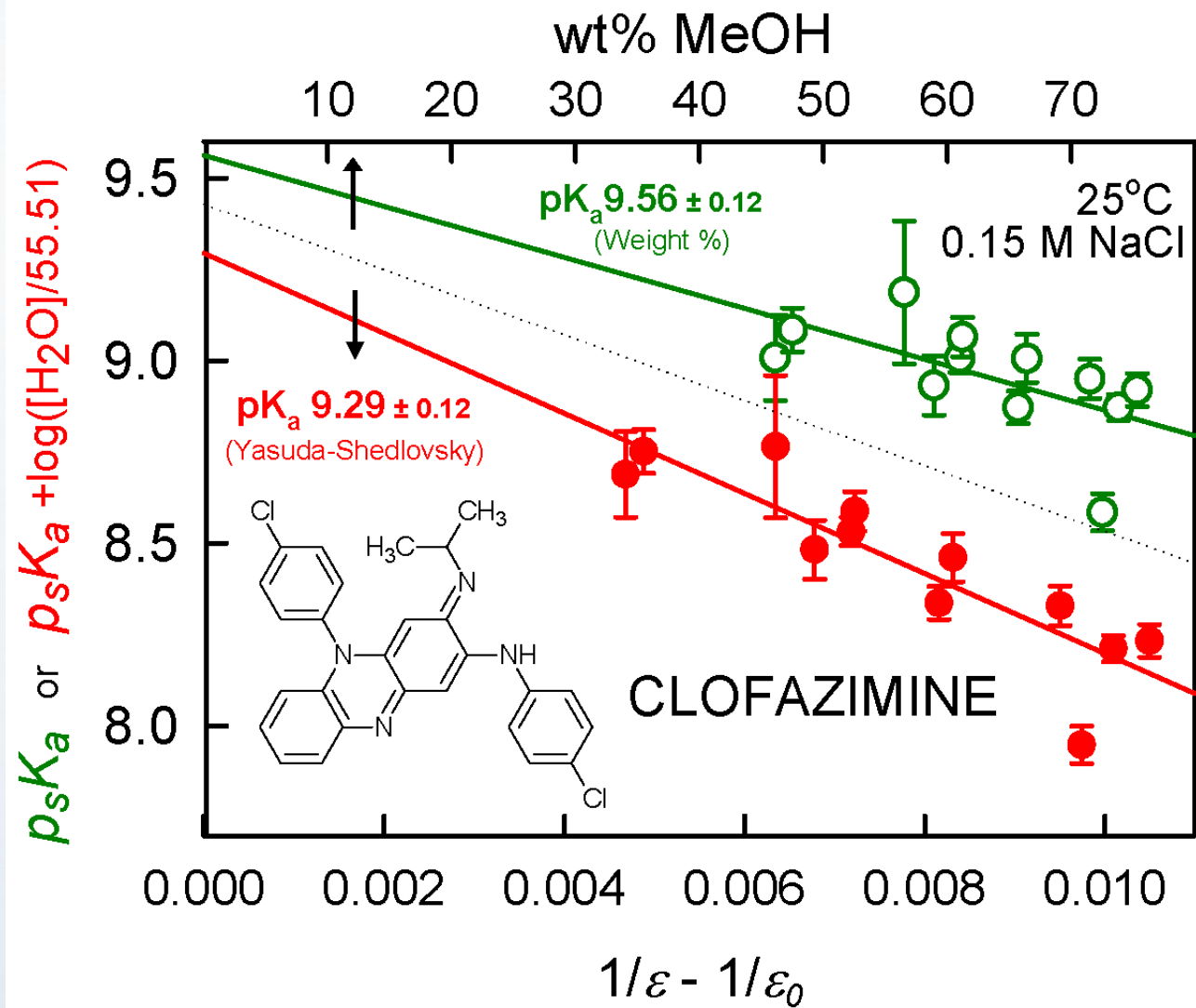
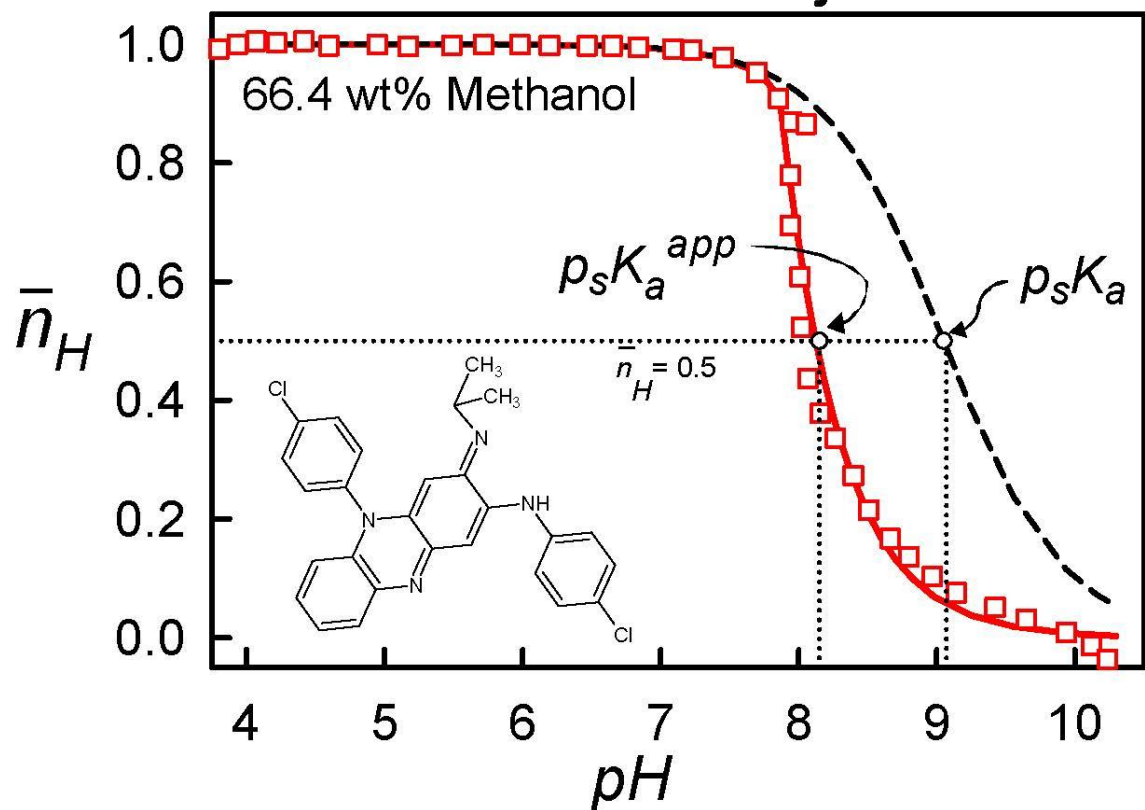


12 titrations

0.2-0.9 mM CFZ

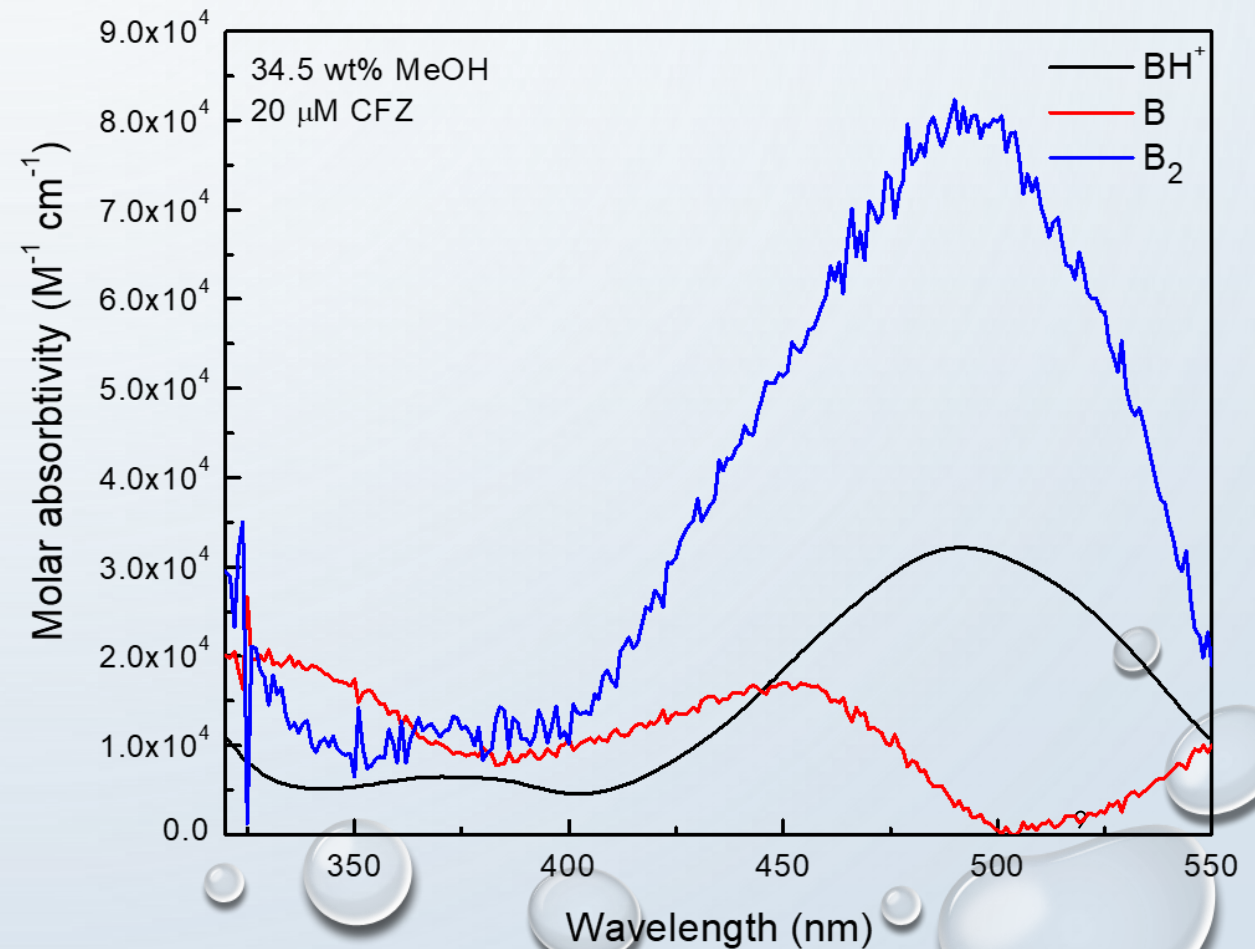
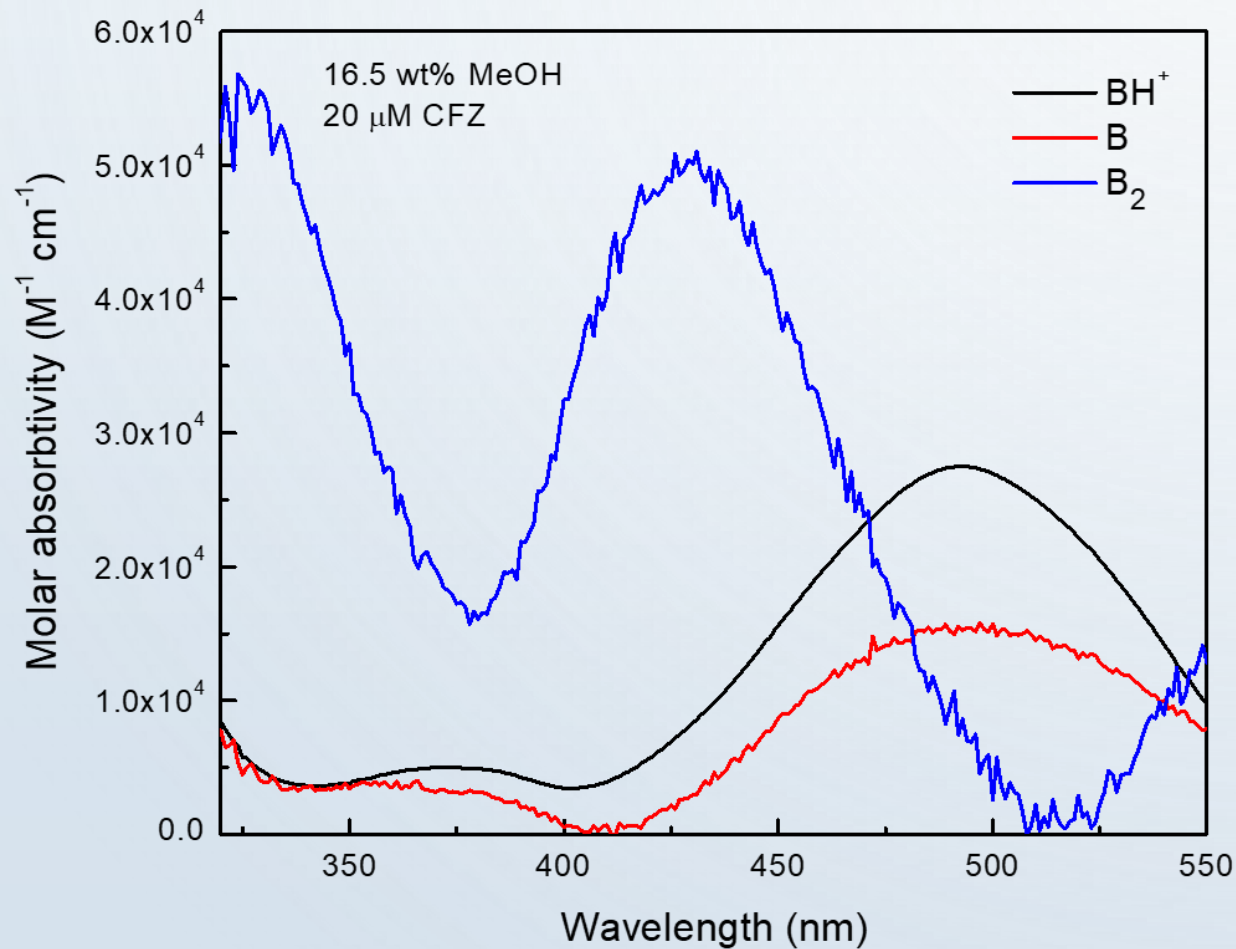
46-75 wt% MeOH

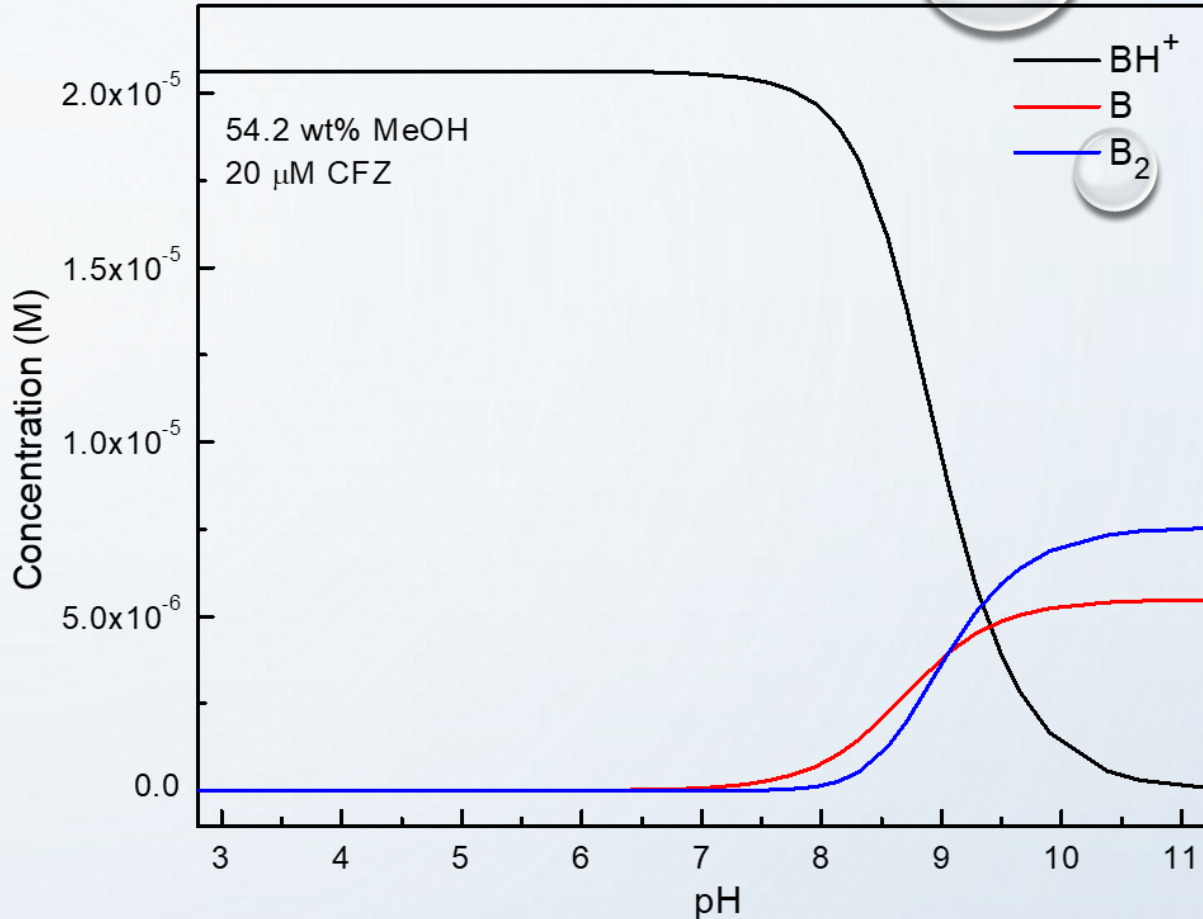
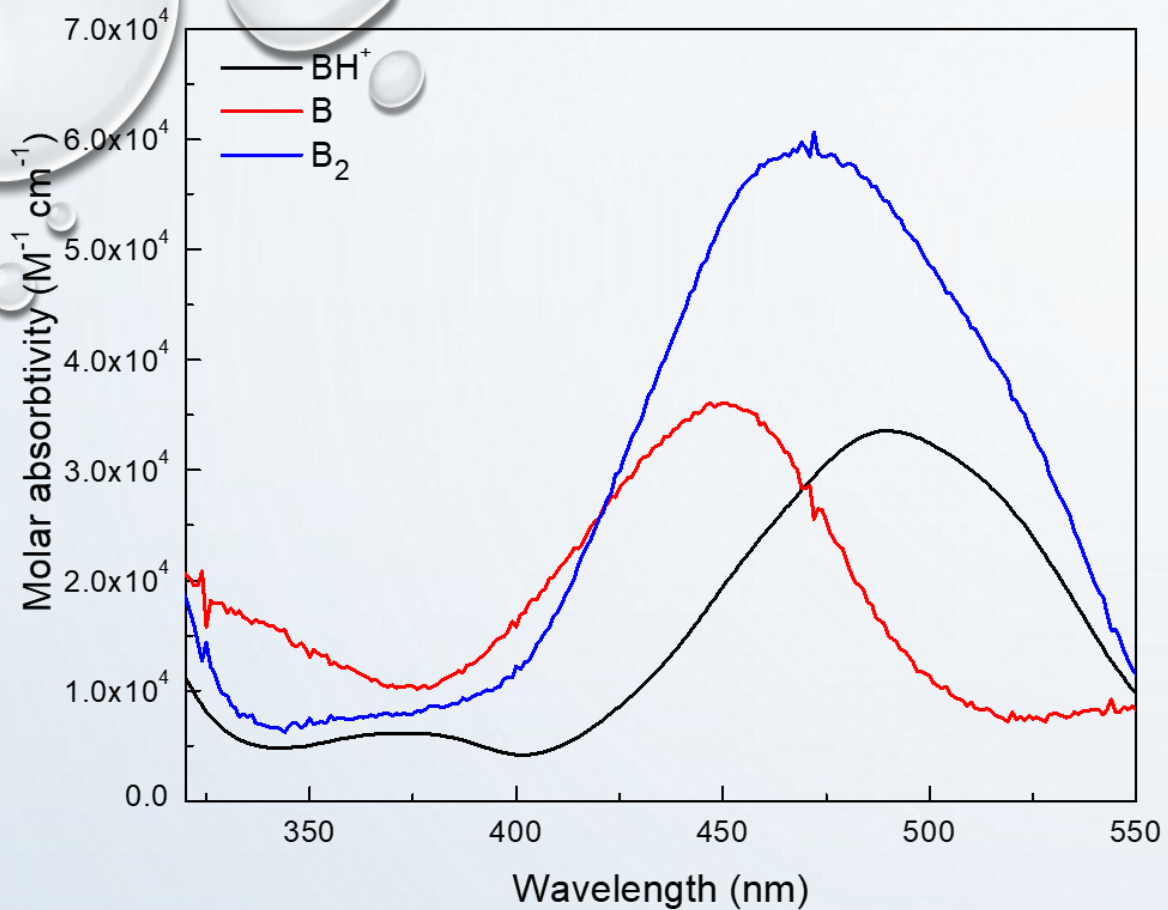
Clofazimine Cosolvent Bjerrum Plot



What if reverse cosolvent pK_a dependence observed by UV/Vis spectrophotometry is an **indicator of the presence of CFZ dimers** in aqueous solutions?

Calculated UV/Vis spectra

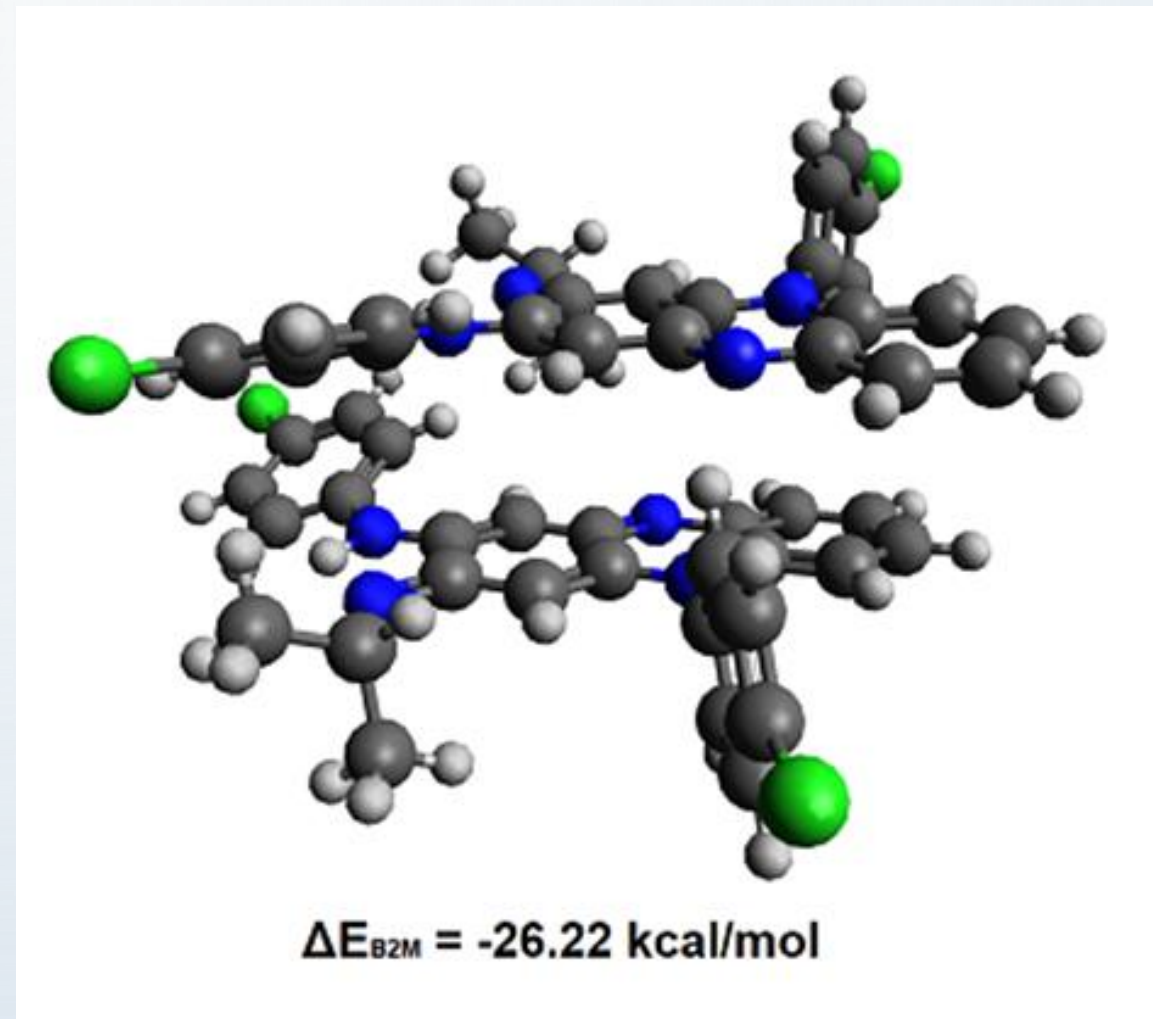
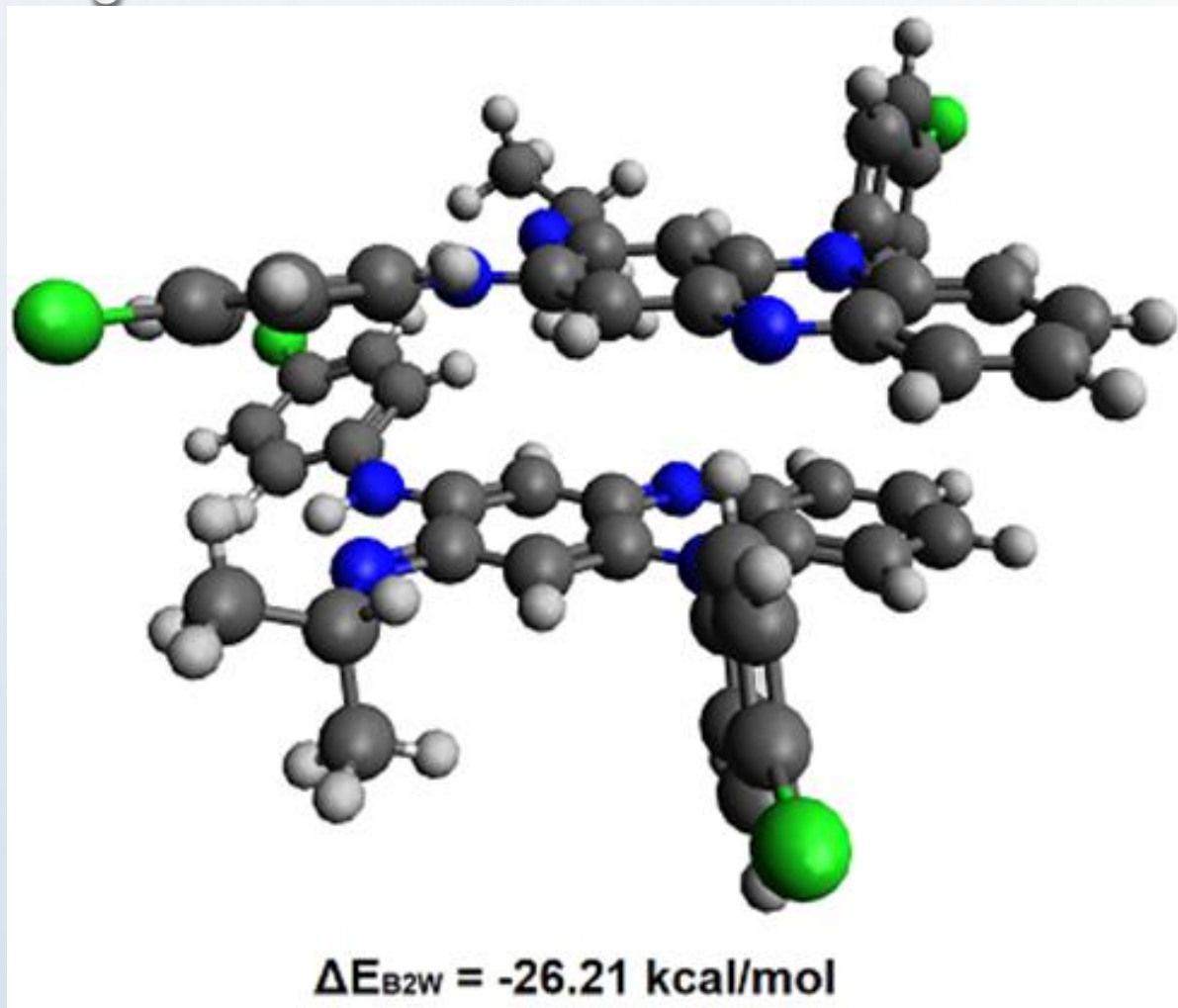




54.2 wt% MeOH		34.5 wt% MeOH		16.5 wt% MeOH	
Log K	$p_s K_a$	log K	$p_s K_a$	log K	$p_s K_a$
5.54 ± 0.30	9.51 ± 0.17	5.02 ± 1.54	8.67 ± 0.38	4.04 ± 1.54	7.91 ± 0.83

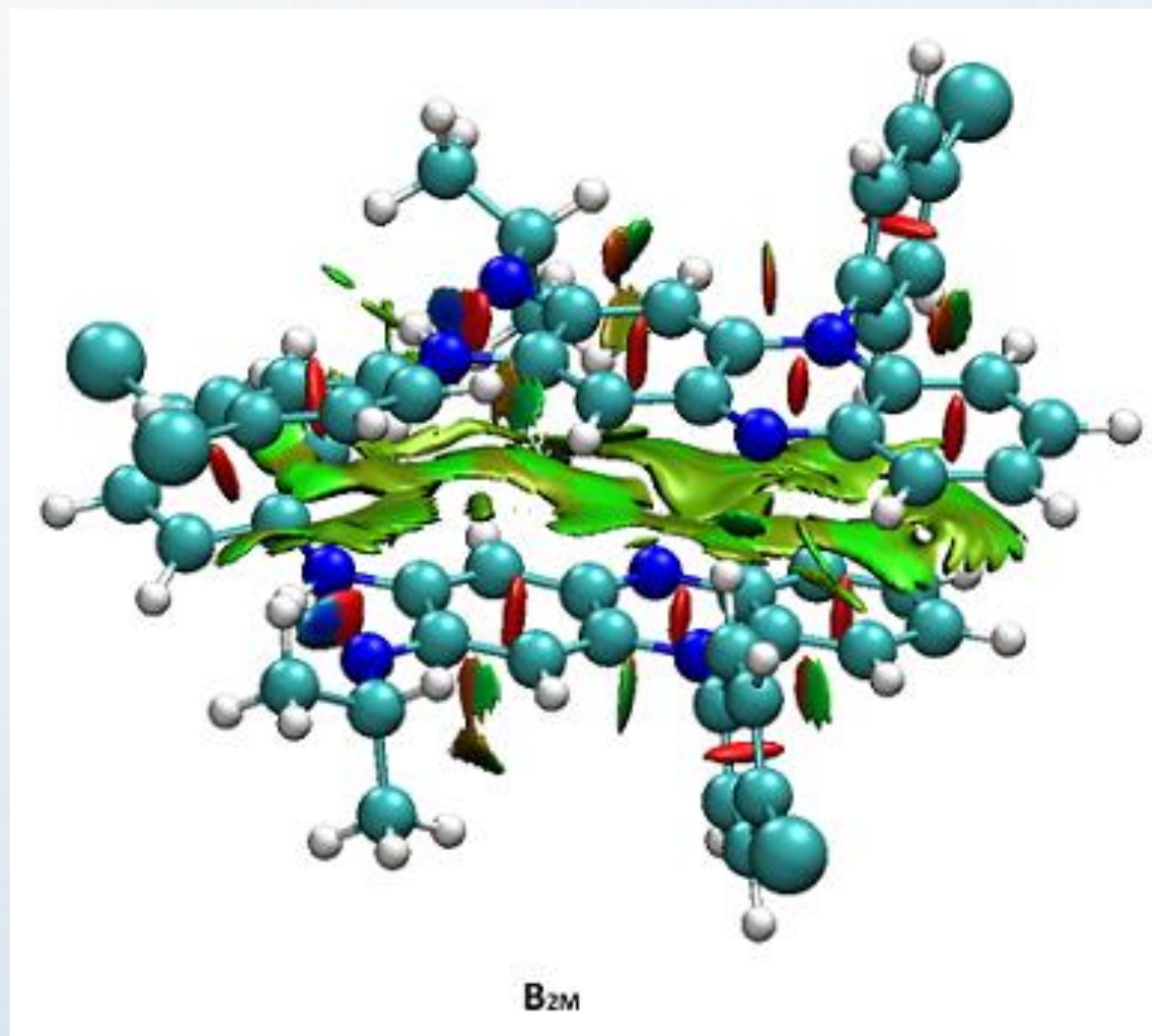
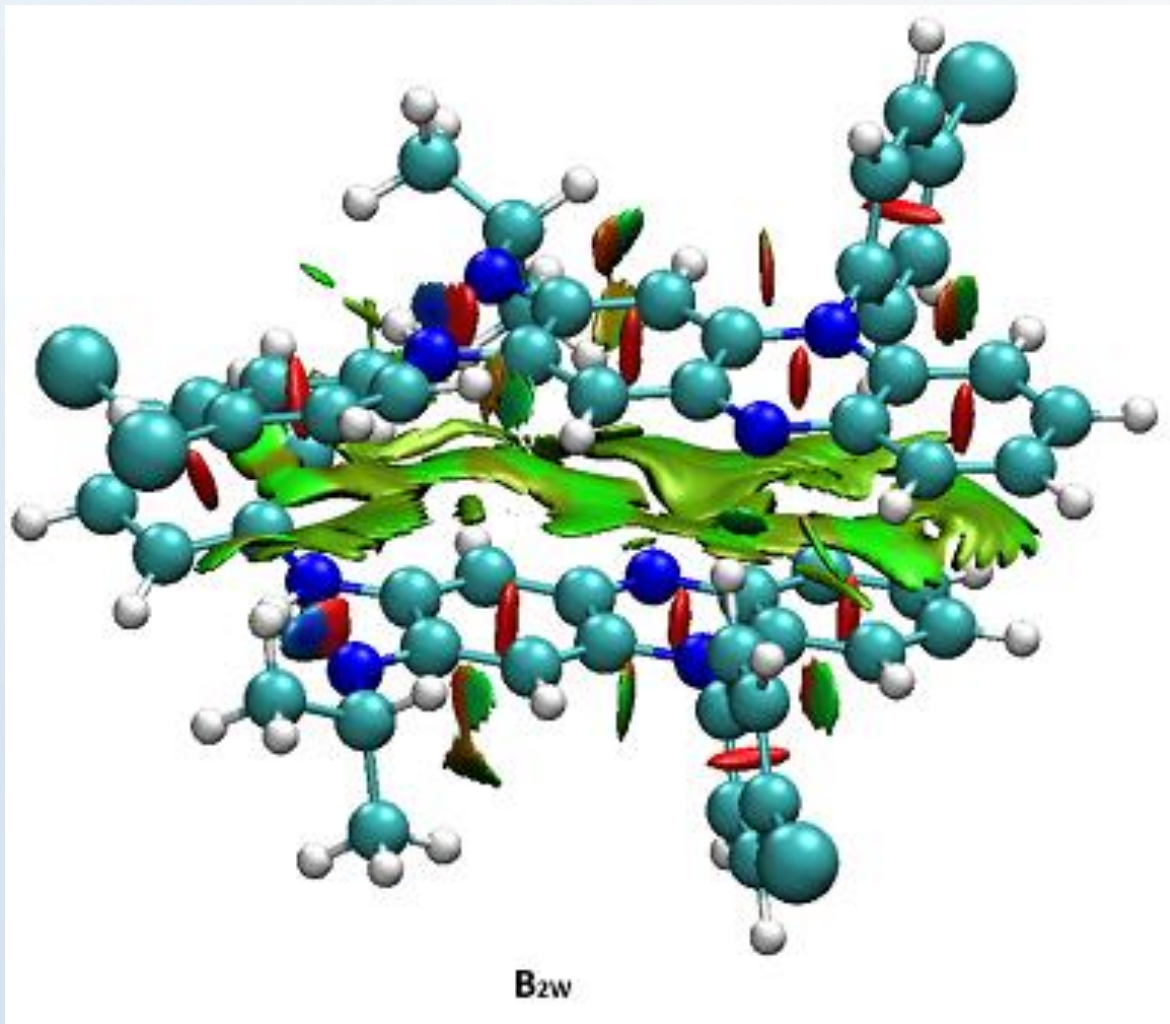
$p_s K_a$ and log K of **CFZ** in MeOH–water co-solvent media (weight MeOH %) as determined by UV/Vis titration and **PCA-ALS** (25±1°C, $I = 0.0$ M)*.

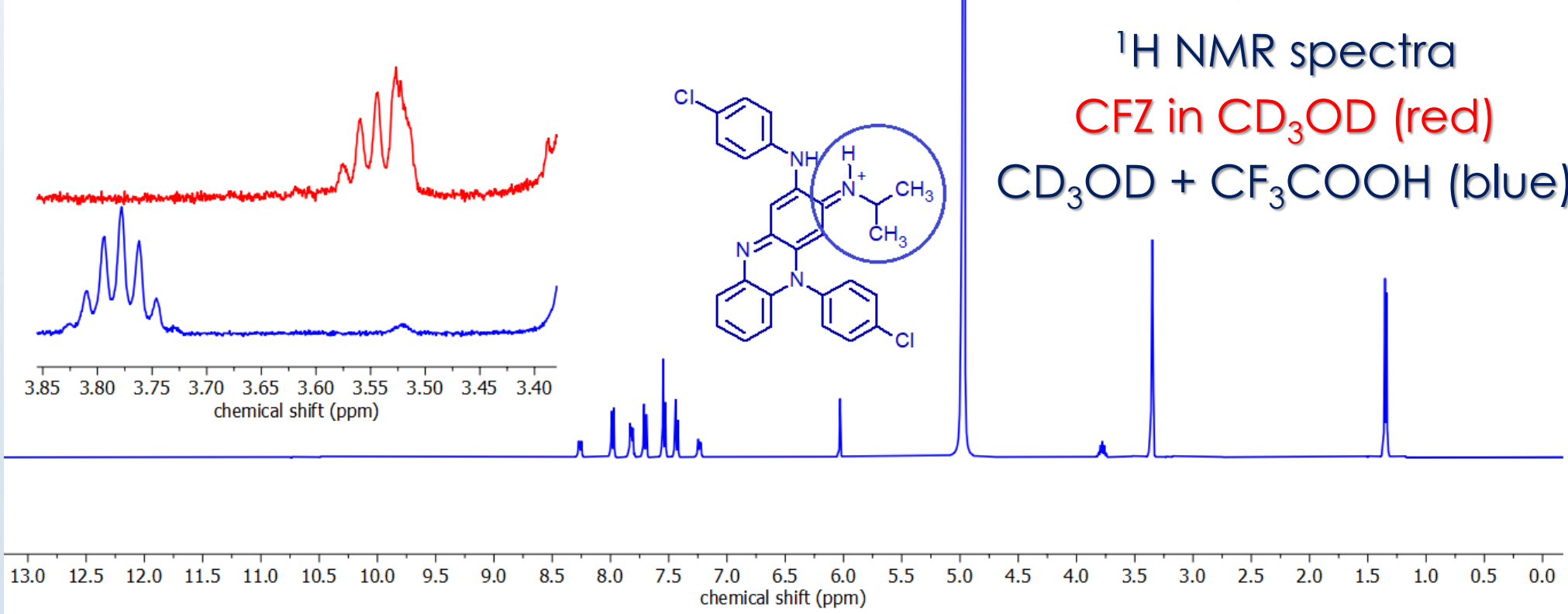
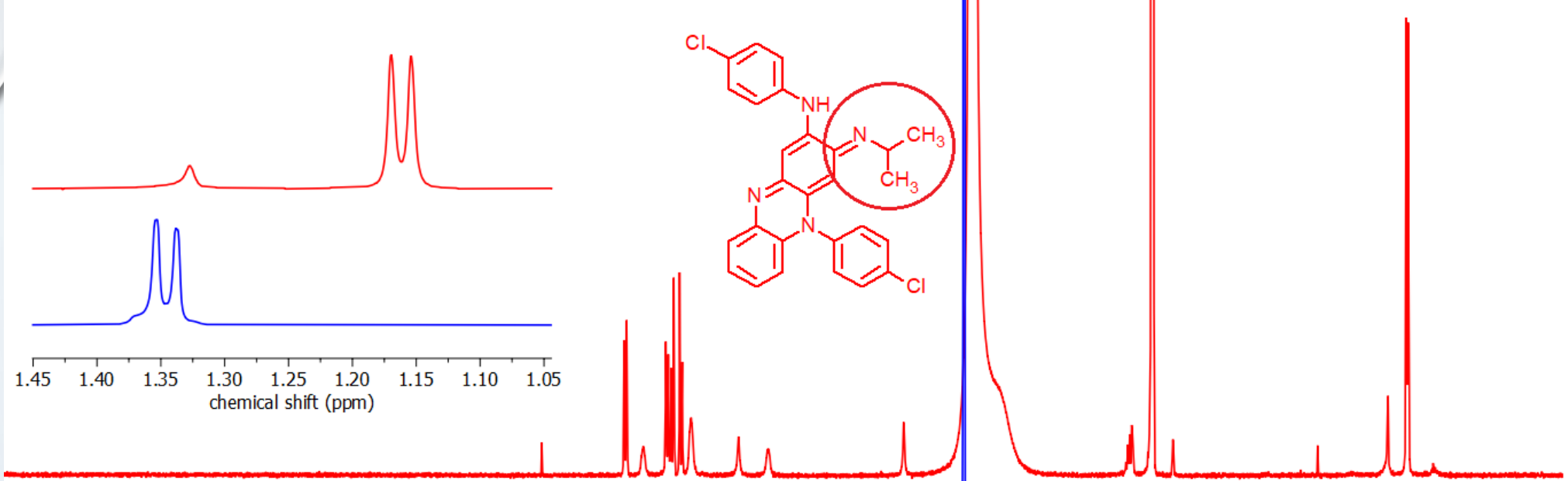
DFT dimerization calculations



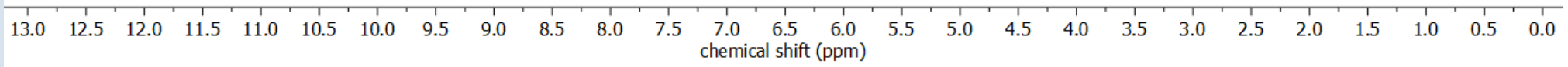
Optimized geometries of B₂ dimers using H₂O (B_{2W}, left) and MeOH (B_{2M}, right) as solvents

NCI plots calculated for geometries of B₂ dimers optimized using H₂O (B_{2W}) and MeOH (B_{2M}) as solvents. Green areas correspond to the attractive non-covalent contacts, while brown/red areas correspond to the repulsive interactions.





¹H NMR spectra
CFZ in CD₃OD (red)
CD₃OD + CF₃COOH (blue)



Clofazimine pK_a Determination by Potentiometry and Spectrophotometry: Reverse Cosolvent Dependence as an Indicator of the Presence of Dimers in Aqueous Solutions

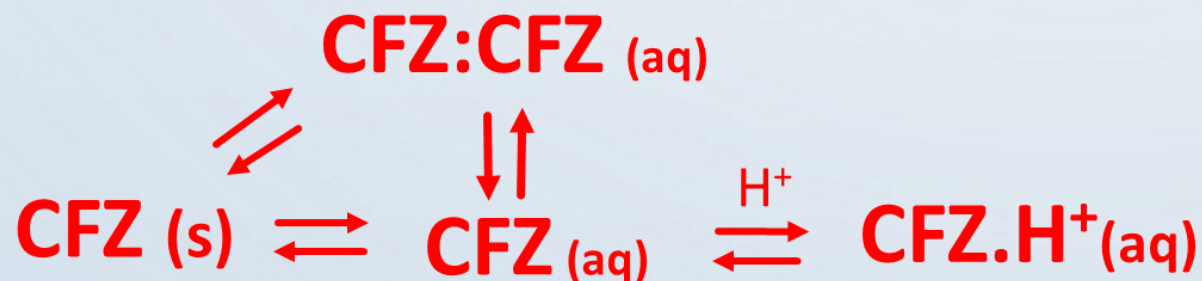
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Cite This: *Mol. Pharmaceutics* 2023, 20, 3160–3169



Read Online



Aqueous pK_a :

9.43 (pot)

9.61 (spec)

St. John's Univ, NYC, December 2021



Thank you all for the attention!