Vibrational features of dacarbazine and deticene revealed by Raman, SERS, THz-Raman and DFT methods

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Introduction

Dacarbazine (DTIC, 5-(3,3-dimethyltriazeno)imidazol-4-carboxamide), is one of the most used antineoplastic chemotherapy compound in treating metastasized malignant melanoma, advanced lymph node cancer (Hodgkin's disease) and advanced cancers of soft tissue (soft tissue sarcomas) [Egg04, Tag08]. The drug is administered through injection (commercially known as Deticene - DET), which is reconstructed from DTIC with citric acid and mannitol at a pH of 3.0-4.0. DTIC is extremely sensitive to light, its biological activity being also greatly affected by the pH of the medium in the biological range 7.0-8.0[Sau86].

Since the protonated and deprotonated nitrogens act as a proton donor or acceptor in hydrogen bonding, interactions of dacarbazine with proteins/nucleic acids may depend on a particular molecular form of dacarbazine. Thus, in order to get further insight into the structure/activity relationship, it is important to understand the precise molecular structure and structural behavior of the drug at different pH values.

Materials and methods

DTIC was purchased from Sigma Aldrich as crystalline powder. Deticene used in this study was a commercial drug supplied by the Faulding company. Bi-distilled water was used to prepare the aqueous solutions,. The Raman spectra of DTIC and DET were recorded from solutions of 1 M and 0.02 M, respectively. To adjust the pH to the desired values, small amounts of HCl, citric acid or NaOH were added.

Hydroxylamine reduced silver colloid was prepared according to the method published by Leopold and Lendl [Leo03]. The SERS spectra at different pH values were recorded by adding 30 mL 10^{-3} M of DTIC or Deticene aqueous solution to 500 mL silver colloid.

Raman and SERS spectra of the solutions were recoded using a Renishaw InVia Raman system using a 532 nm diode pumped solid state (DPSS) laser with a maximum power of 200 mW and a grating with 1800 lines/mm. For the acquisition of the ultra-low frequency Raman spectra we used the Near Excitation Tunable (NExT®) filter from Renishaw plc.

As starting geometries for energy minimizations, we used the X-ray structures derived from crystallographic data reported by Freeman and D. Hutchinson [Fre79]. The calculations were performed at B3LYP/6-31+G(2d,2p) level of theory, using the Gaussian 09 package [Gau09]. Solvent effects have been considered by using the PCM continuum model [Men02].
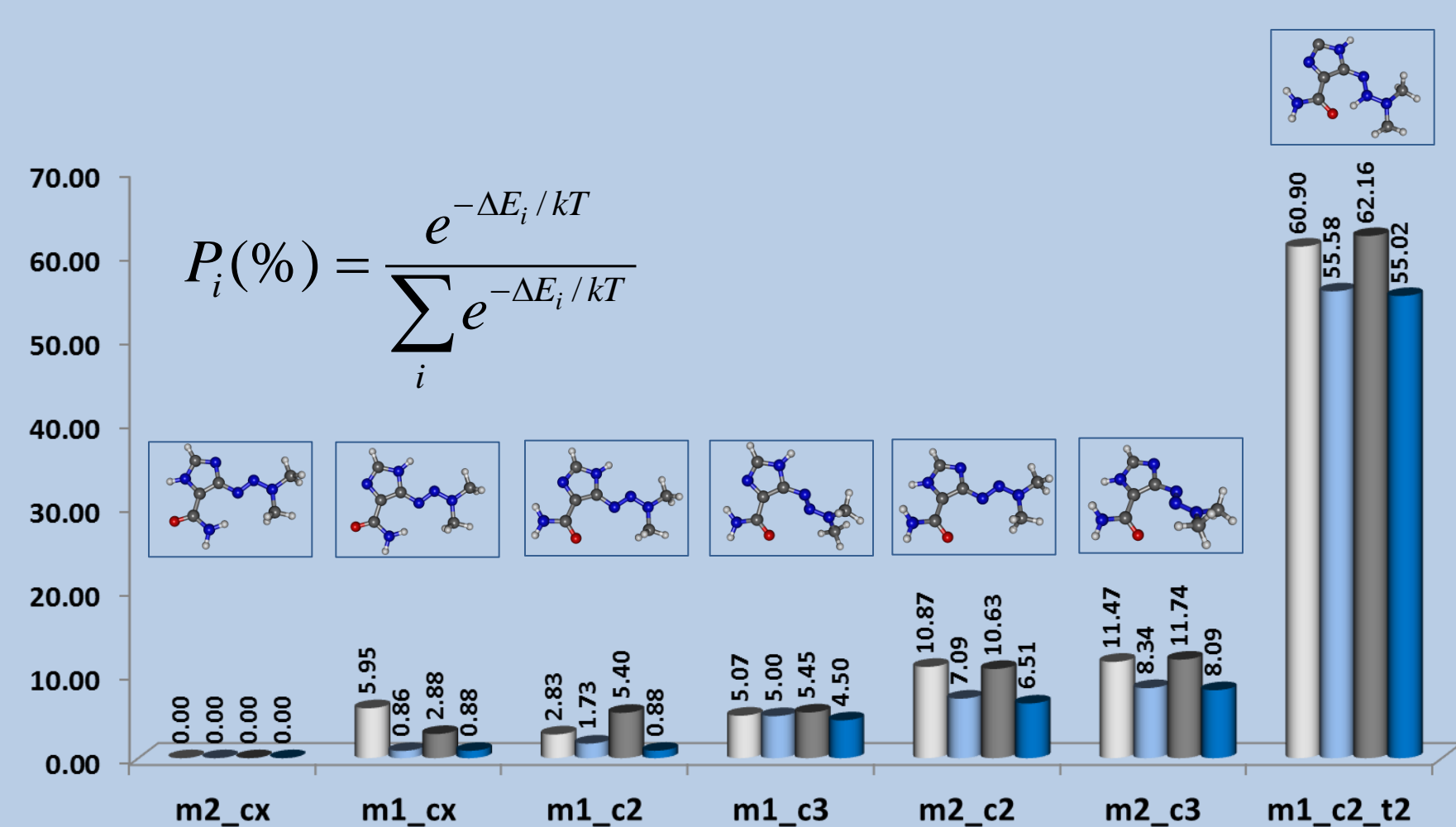
Results and Discussion

Dacarbazine's conformers

$$P_i(\%) = \frac{e^{-\Delta E_i/kT}}{\sum_i e^{-\Delta E_i/kT}}$$

Fig.1 Relative energies (ΔE_{HF} – light color, ΔG – dark color) of the DTIC monomers calculated at B3LYP/6-31+G(2d,2p) level of theory, in gas (grey) and water (blue)

Conformer	m2_cx	m1_cx	m1_c2	m1_c3	m2_c2	m2_c3	m1_c2_t2
Relative Gibbs free energy in water (kcal/mol)	0.00	0.88	0.88	4.5	6.51	8.09	55.02
Boltzmann population at RT (%)	69.14	15.41	15.41	0.03	0.00	0.00	0.00

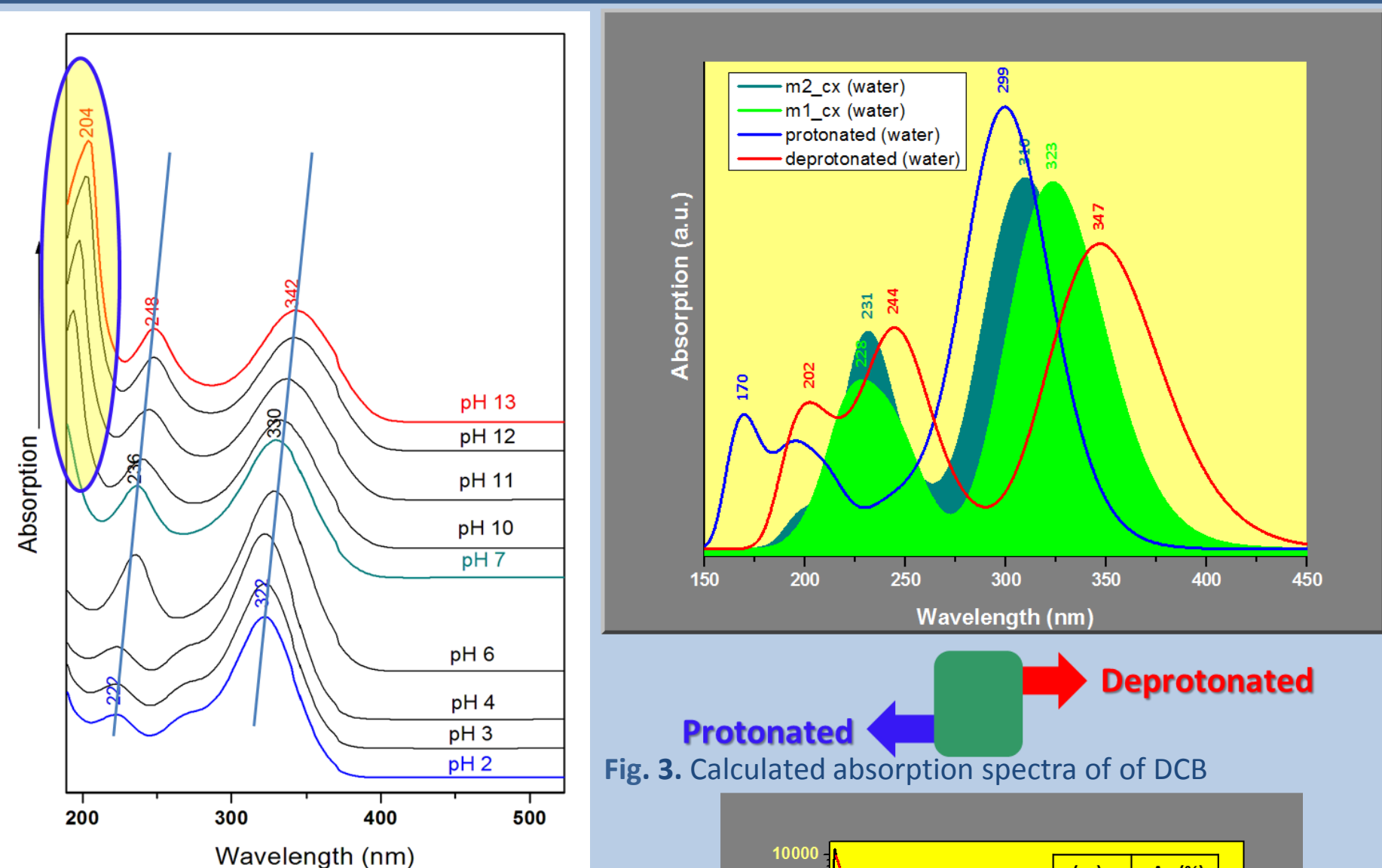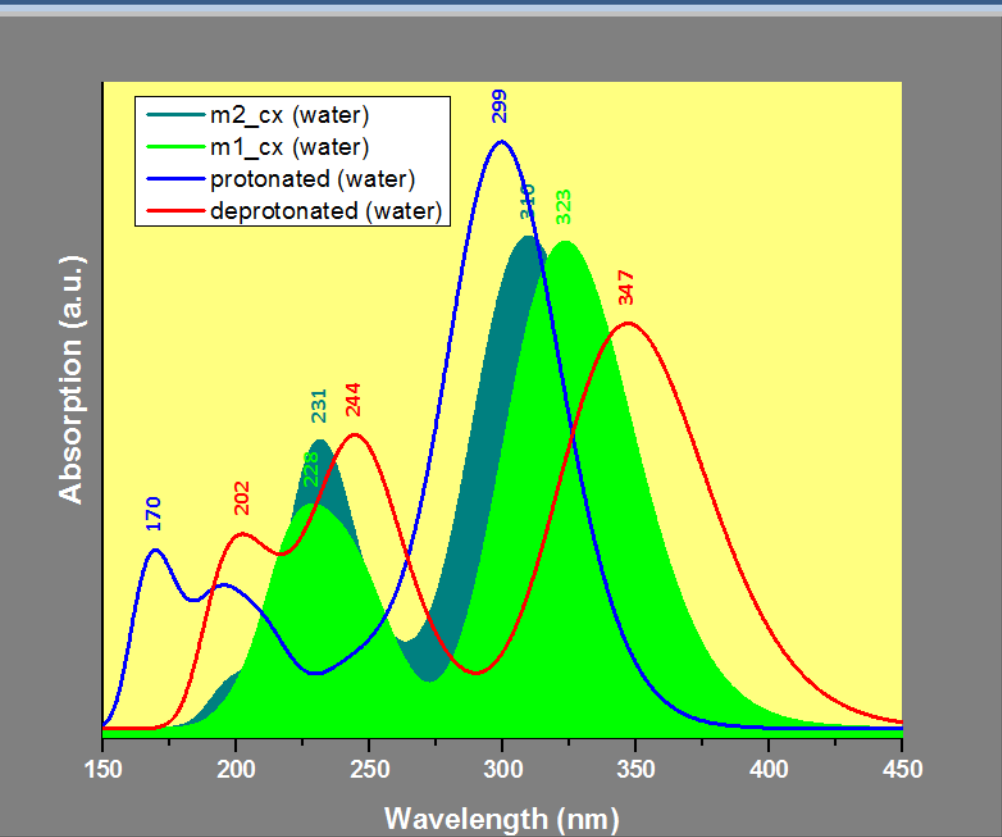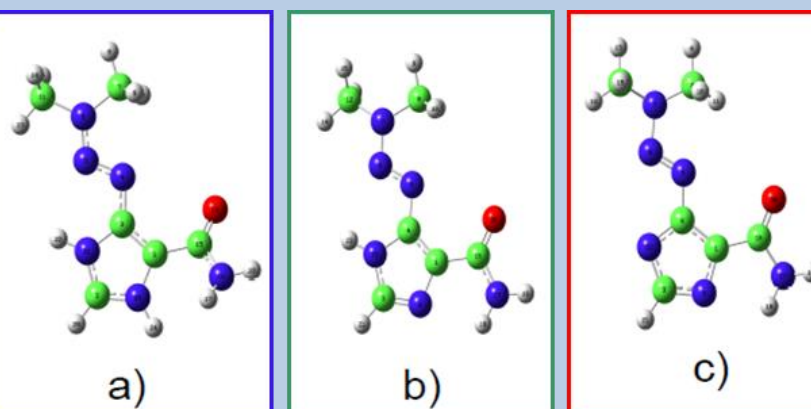### Absorption spectrum of Dacarbazine

Fig. 2. pH dependence of the absorption spectra

Fig. 3. Calculated absorption spectra of of DCB

Protonated ⟷ Deprotonated

Fig. 4. Time-resolved fluorescence at of $0.8 \cdot 10^{-2}$ M aqueous solution Dacarbazine (excitation: 375, nm laser power 28.3 μW)

τ(ns)	<A> (%)
0.62	64.60
1.27	30.05
4.62	4.90

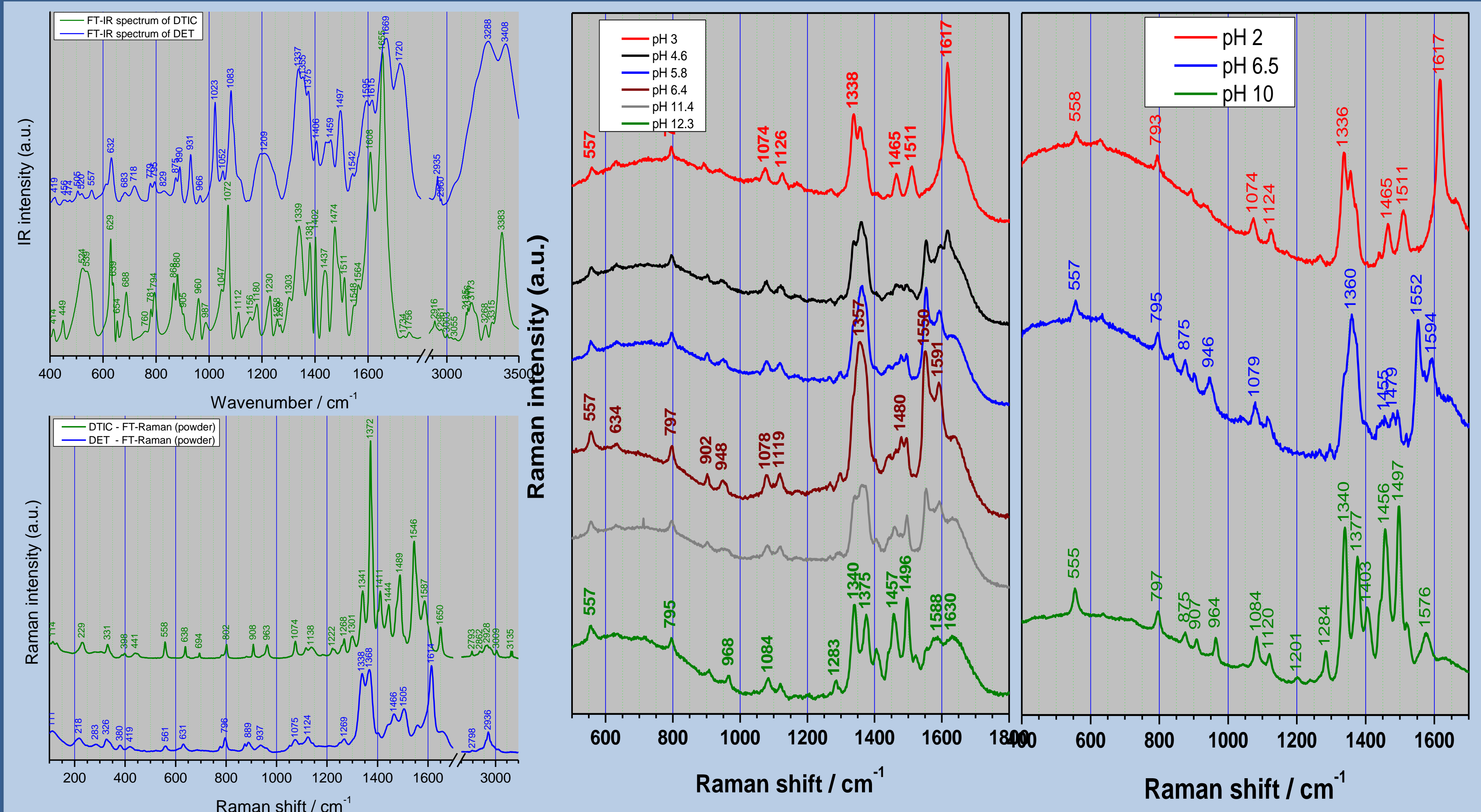## Infrared, Raman and SERS spectra of dacarbazine and deticene

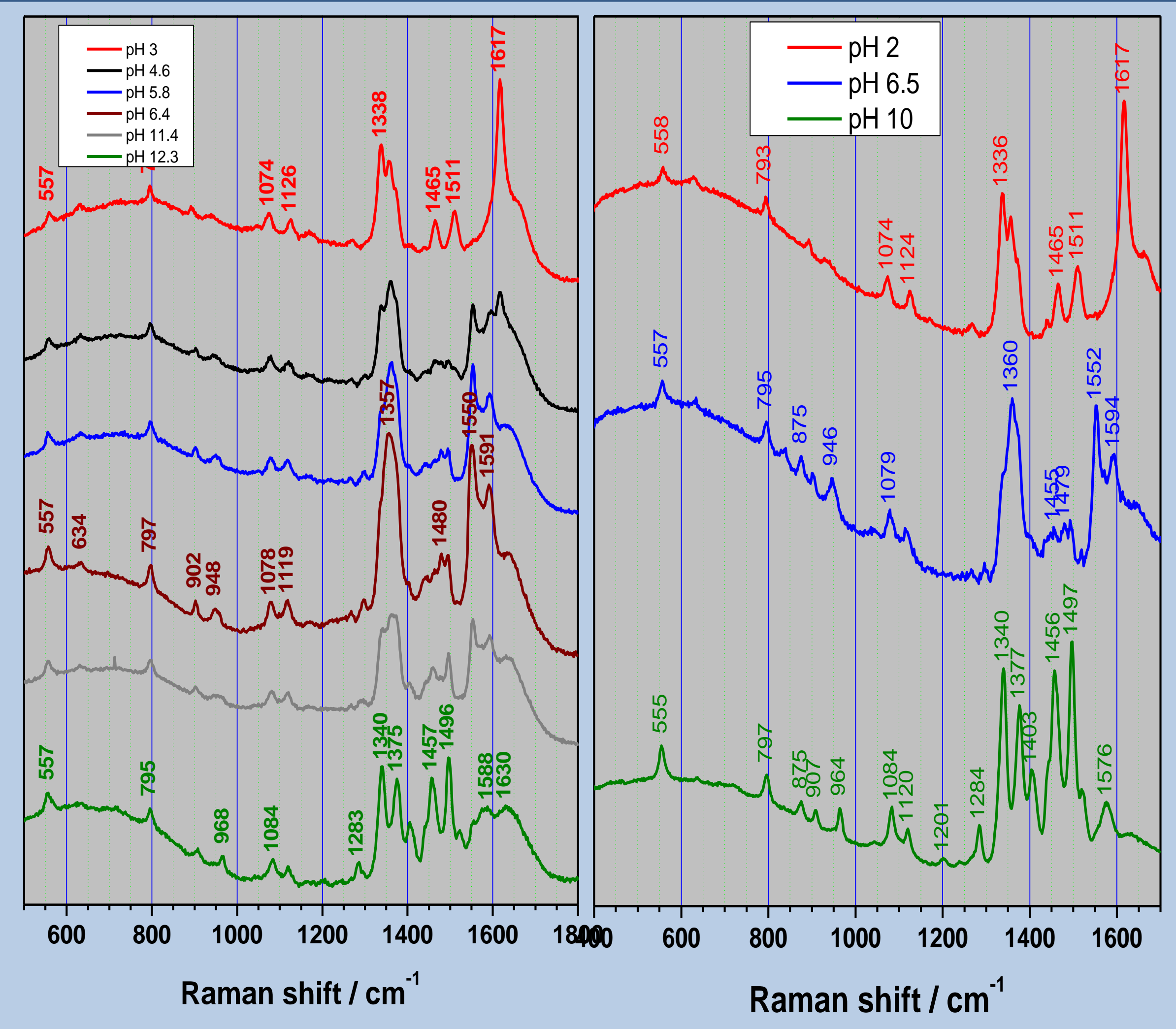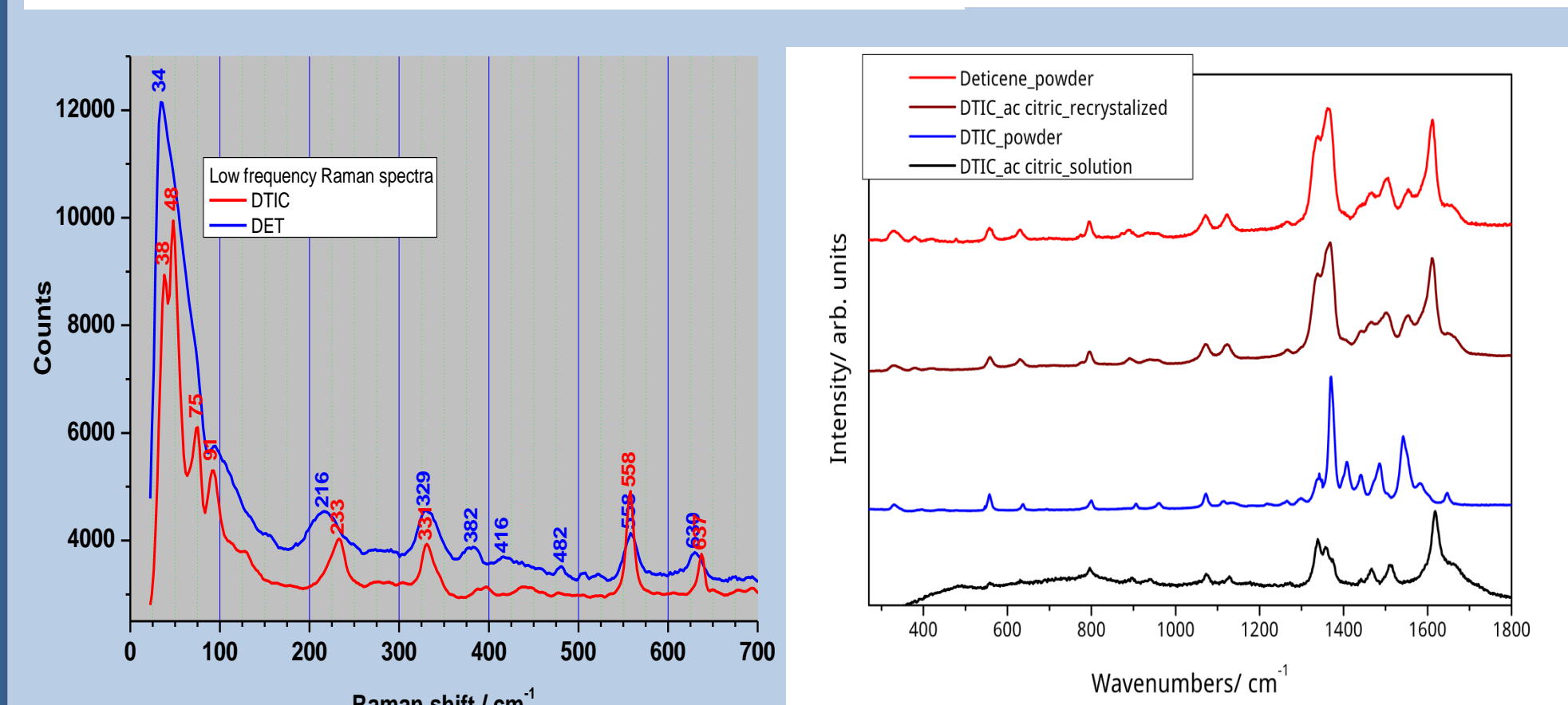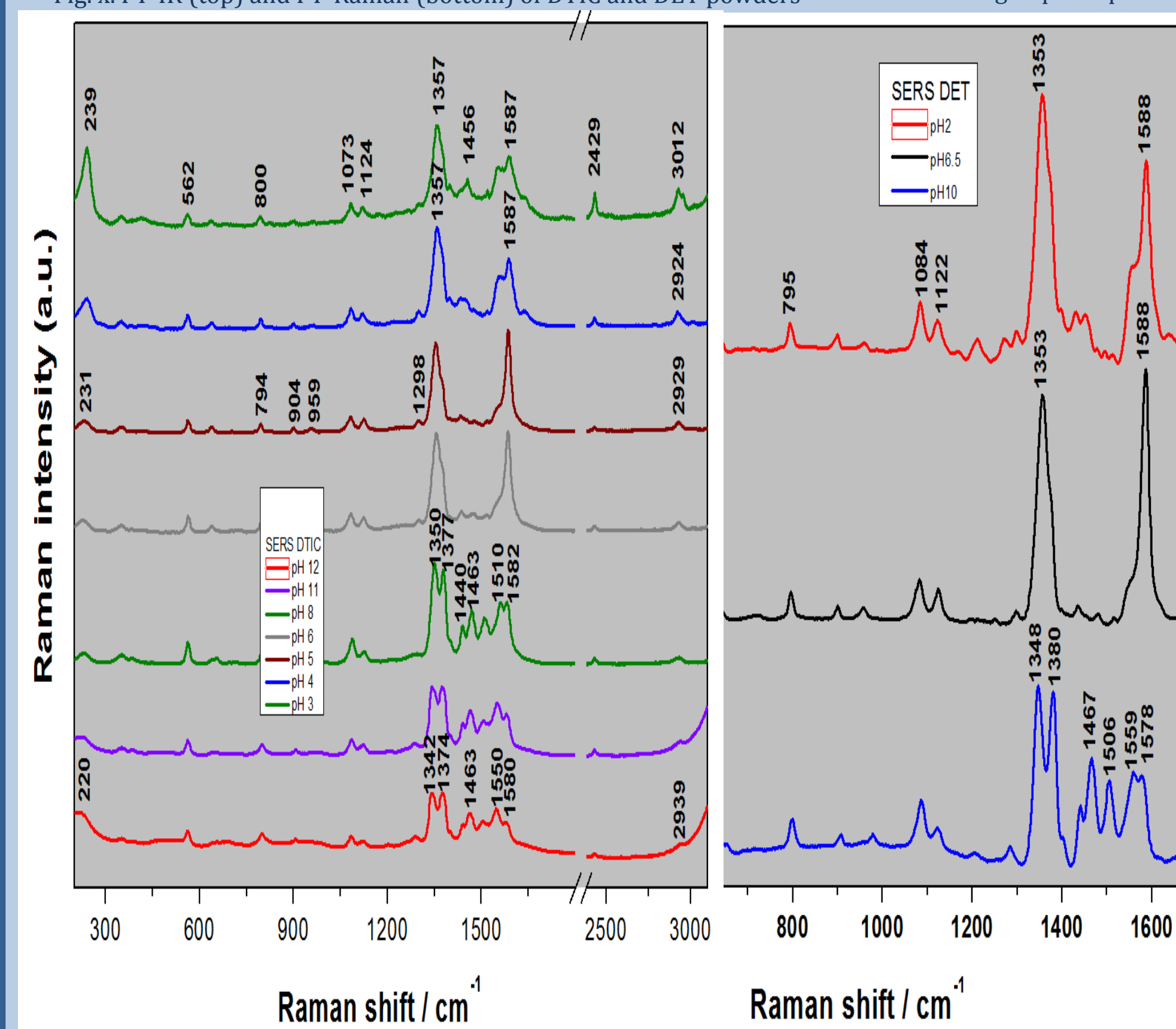Fig. x. FT-IR (top) and FT-Raman (bottom) of DTIC and DET powders

Fig. x. pH-dependence of Raman spectra of DTIC (left) and DET (right) in water solution at room temperature

Experimental data		Computed data				
DTIC FT-IR/ FT-Raman	DET FT-IR/ FT-Raman	DTIC FT-IR/ FT-Raman	DET FT-IR/ FT-Raman	Assignment**	Functional group	Comments
3383/-	3408/-	3434/3436	3574/3574	$v_{as}(NH_2)$	Am	shifted between DTIC and DET
3268/-	3288/-	3265/3267	3417/3415	$v_s(NH_2)$	Am	characteristic for DET
3135/3135		3130/3132		$v(NH)$ (H-bonded)	Im	characteristic for DTIC
-/2927	2935/2936	2934/2933	2941/2941	$v_s(CH_3)$	Me	characteristic for DET
1655/1650	1720/- 16691657	1667/1664	1692/1692 1657/1657	$v(C=O)+ \delta(NH_2)$	Am	blue-shifted for DET
1608/1607	1615/1595/ 1614	1596/1598	1567/1567	$\delta(NH_2)+v(CO)$	Am	blue-shifted for DET
1548/1546	1595/1592	1531/1531	1591/1591	$v(C-C)+\delta(NH)$	Im	intensity ratio well reproduced for DTIC
1511/1509	-/1505			$\delta(CH_3)+v(NN)+$ Im ip. def.	Me+Tr+Im+ Am	characteristic for DET
1491/1489	1497/-	1492/1491	1476/1474	$\delta(CH_3)+v(NN)+$Im ip. def.	Me+Tr	characteristic for DET
1474/1473	1459/1466	1452/1461	1450/1448	$\delta(CH_3)+v(NN)+$Im ip. def.	Me+Tr+Im	red shifted for DET
1381/1372	1373/1368	1384/1387	1374/1373	$v(NN)+ u(CH_3)+$Im ip. def.+ $\delta(OCN)$	Am+Tr+Im+ Me	1373 and 1357– doublet characteristic for DET in FT-IR and Raman
1339/1341	1337/1339	1344/1357	1338/1357	$v(NN)+\delta(CH_3)+$ $\delta(MeNMe)$	Tr+Me+Im	
1230/1230	1209/-	1221/1221	1192/1191	Im ip def.+$p(NH_2)$ $+\delta(CH)+\delta(NH)$	Im+Am	characteristic for DTIC broad IR band for DET
1072/1074	1083/1075	1059/1061	1050/1049	$\delta(CNC)$ Im + $\delta(MeNMe)+p(CH_3)$	Im+Tr+Me	blue-shifted in FT-IR for DET
-/-	1023/-	-/-	1025/1025	$\delta(NNN)+p(CH_3)$	Tr+Me	characteristic band for DET
960/963	967/962	948/948	921/922	$\gamma(NH)+\delta(NCN)$	Im	characteristic for DTIC
905/908	931/937	923/926	881/880	$\gamma(NH)-$ $\gamma(CH)+\delta(NNN)$	Im+Tr	blue-shifted, characteristic for DET
688/694	682/-	705/688	707/675	$\delta(OCNH_2)+$ Im ip. def.	Am+Im	characteristic for DTIC
639/638 629/-	-/- 632/631	642/649	634/639	p (Im.)+$\delta(NNC)$	Im+Tr	doublet characteristic for Im [Soc04]
558/558	557/561	562/562	550/550	$\delta(CCNH_2)+\delta(NNN)$	Am+Tr	characteristic for DTIC
539/- 524/-	522/-	527/529	284/279	$\omega(NH_2)$	Am	doublet characteristic for DTIC
449/441	-/-	451/441	447/447	$\delta(CCN)$ Tr $+\delta(MeNMe)$	Tr+Me	characteristic for DTIC

Conclusions

- Both, in gas-phase and water solution, the m2 tautomer is the most stable.
- Unlike the solid state, the spectroscopic response of DTIC in water solution must be explained considering the contribution coming from both tautomers, m2_cx and m1_cx.
- Proper model and solvent effects must be considered for a reliable assignment of UV-Vis spectra. Excellent agreement between the experimental UV-Vis data and TD-DFT results
- Fluorescence lifetime experiment suggest the presence of three different species in aqueous solution of DTIC
- Different molecular species of Dacarbazine have been identified in acidic, neutral and basic water solutions. Characteristic bands are:
  protonated : Raman - 1617 and 1356 cm^{-1}, SERS – 1587 and 1557 cm^{-1}
  neutral : 1592, 1553 and 1357 cm^{-1}, SERS – 1587 and 1357 cm^{-1}
  deprotonated : 1496, 1456 cm^{-1}, SERS – 1550, 1463 and 1342 cm^{-1}
- DET is obtained from citric acid solution of DTIC, by recrystallization
- Similar species have been detected adsorbed on Ag colloids for DTIC and DET and the same adsorption geometry is concluded
- Detection limit of DTIC: Raman - 10^{-3} M, SERS - $9.09 \cdot 10^{-7}$ M
- Characteristic low frequency bands for DTIC: 38, 48, 75 and 93 cm^{-1}

References

[Tag08] Tagne J.-B., Kakumanu S., Nicolosi R.J., Mol. Pharm., 5 (2008) 1055–1063.
[Egg04] A.M.M. Eggermont, J.M. Kirkwood, Eur. J. Cancer, 40 (2004) 1825–1836
[Sau86] Saunders P., DeChange W., Chao L., Chem.-Biol. Int., 58 (1986) 319-331.
[Bal79] Balsamo A, Crotti P, Lapucci A, Macchia B, Macchia F, Del Tacca M, Mazzanti L,
[Fre79] H.C. Freeman and D. Hutchinson, Acta Cryst., 1979, B35, 2051-2054.
[Leo03] N. Leopold, B. Lendl, J. Phys. Chem., B. 107 (2003), 5723–5727.
[Men02] B. Mennucci, J. Tomasi, R. Cammi, J.R. Cheeseman, M.J. Frisch, F.J. Devlin, S. Gabriel, P.J. Stephens, J. Phys. Chem. A 2002, 106, 6102-6113.
[Gau09] Gaussian 09, Revision A.02, M. J. Frisch et al., Gaussian, Inc., 2009.
[Fre79] H.C. Freeman and D. Hutchinson, Acta Cryst. B35, (1979) 2051-2054.

Acknowledgments