ANTI-CANCER DRUG (5-FLUOROURACIL) BEHAVIOR IN VIBRATIONAL SPECTROSCOPY

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Keywords: *5-fluorouracil, micro-Raman spectroscopy, SERS, DFT calculations*

Abstract: The micro-Raman and SERS spectra of the well-known anticarcinogenic drug 5-fluorouracil in combination with DFT calculations allowed us to identify and distinguish between the biologically essential enol and enolate forms, and their corresponding Ag-complexes.

The well-known anticarcinogenic drug 5-fluorouracil (5-FU) is a fluorinated pyrimidine antimetabolite, which is mostly employed in palliation of inoperable malignant neoplasms [1]. Moreover, all uracil compounds, in which the hydrogen bounded at the C5 atom is substituted by halogen atoms (such as F, Cl, Br, or I), are presently tested against HIV [2,3] and used as antitumor [4] and antiviral [3] drugs.

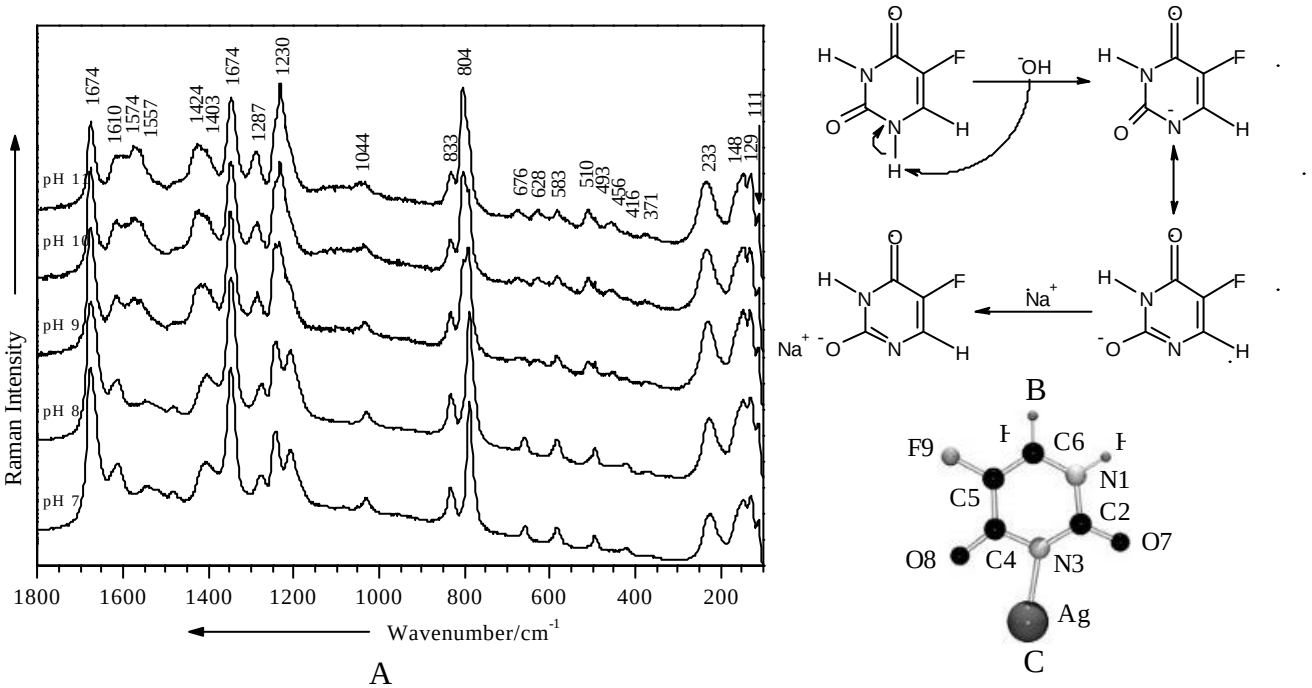

Fig. 1. A: SERS spectra of 5-FU on Ag colloid at neutral and basic pH values. Excitation line: 514.5 nm.
B: The mechanism for the formation of the enolate ions.
C: Orientation model for the N3-deprotonated species of 5-FU on the Ag surface (B3LYP/LANL2DZ calculated structure).

It is well known that the pK_a value of free 5-FU is about 7.8 [5]. But we cannot rule out the possibility that the pK_a value of 5-FU may change with the environment. For example, 5-FU was found to form only wobble base pairs with guanine even under basic conditions (pH = 9) in the crystal lattice [6]. This is a very surprising result because at pH value of 9 a significant portion of the molecule in solution should exist in the ionized form, which can pair with guanine using two H-

bonds, in the well-known Watson-Crick geometry [6]. Such changes in the stacking geometry could be connected with the mutagenic action of 5-FU.

In order to identify and further investigate the 5-FU species present at different pH values or concentrations, the Raman and SERS spectra of 5-FU in water solution and near a biological artificial model (a Ag colloid – partially shown in Fig. 1A) were recorded for the first time and discussed with the help of specific DFT-calculations (harmonic vibrational wavenumbers, Raman scattering activities, natural population analysis (NPA), total electron density).

It was found that at acid pH values the carbonyl bond is protonated. The oxygen atom acts as a Lewis base taking the H-atom from the chloric acid and regenerating an intermediate cation, which can be represented by two resonance forms. The most stable intermediate cation loses its proton and leads to the enol form (not shown here). At basic pH values, the base deprotonates the N1-H bond, leading to an intermediate enolate ion, that is again represented by two resonance structures. The Na^+ addition to the oxygen atom yield the neutral enolate (shown in Fig. 1B). The micro-Raman spectra allowed us to experimentally identify and distinguish between these forms.

After a detailed analysis of the SERS spectra at acid pH values, the molecule was found to be chemisorbed on the metal surface through the deprotonated N1 form and one or both oxygen atoms (as the NPA analysis showed), more probably through the lone pair of the O7 atom, in an end-on orientation. But the contribution of the electromagnetic mechanism is also significant. At alkaline pH, both deprotonated forms of the 5-FU are present in the solution and both tautomers adsorb on the silver surface via the N atoms in an upright orientation. The N3 deprotonated form seems to be the dominant tautomer in the adsorbed state (shown in Fig. 1C) because of the smaller delocalisation of the negative charge and also due to a higher dipol moment.

The DFT calculations performed at the B3LYP/LANL2DZ level have aided to see which of the tautomers (shown in Fig. 2) and Ag-complexes are the most stable and to interpret the spectra.

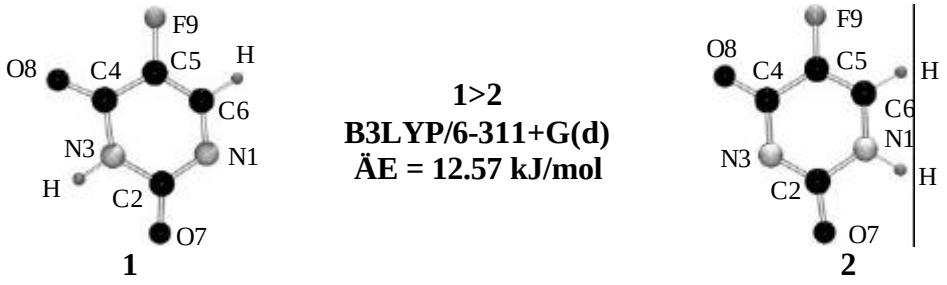

Fig. 2. B3LYP/6-311+G(d) optimized geometries of the two deprotonated forms of 5-FU.

Acknowledgements:
We highly acknowledge the financial support from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich SFB 630, Teilprojekt C1) as well as from the Fonds der Chemischen Industrie.

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