

SECTION 10.10

SATURATION RAMAN SPECTROSCOPY

J. Teraoka, P. A. Harmon and S. A. Asher*

Department of Chemistry, University of Pittsburgh,
Pittsburgh, PA 15260, USA

The high power fluxes associated with the pulsed nature of UV excitation sources can lead to processes which complicate UV resonance Raman (RR) spectral analysis of ground state species. For example, the saturation of the ground state Raman intensities can occur if the ground state population is significantly depleted during the laser pulse. The measured ratios of the analyte Raman intensity to the non-saturated internal standard then become a function of laser power.

Raman saturation, however, can be used to monitor the depletion and repopulation of ground state species during the time interval of the laser pulse. We have developed models for saturation mechanisms, and explicitly explore the RR intensity dependence upon the incident laser power flux to learn about excited state interactions and relaxation processes.

We present here as an example a comparison of saturation between tryptophan-tyrosine dimer (dimer) and the stoichiometric mixture of monomers (monomer). The absorption spectrum of the dimer shows a 20% hypochromicity at 225 nm. Figure 1 shows Raman spectra of monomer (A and B) and dimer (C and D) excited at 225 nm at high (A and C) and low (B and D) power fluxes. The relative intensities of the 1615 cm^{-1} (tyr) and 760 cm^{-1} (trp) bands to the ClO_4^- internal standard band are plotted as a function of the incident laser power flux in Fig. 2. The solid curves in Fig. 2 are least square best fits to a saturation model based on a rate equation which describes the ground state population as a function of time during the laser pulse. The rate equation utilizes the measured absorption cross section and power flux, and incorporates the RR cross section and excited state lifetime as variables.

The conclusions are: (1) In systems where saturation is significant, accurate RR cross sections can be obtained by the fitted curves at zero power flux (2) the relative contributions of both the trp and tyr residues of the dimer to the total observed hypochromicity can be estimated (3) the lifetime of the dimer in the excited state is shorter than that of the monomers due to interactions between the aromatic portions of the dimer residues as well as differences in intermolecular interactions with the solvent bath.

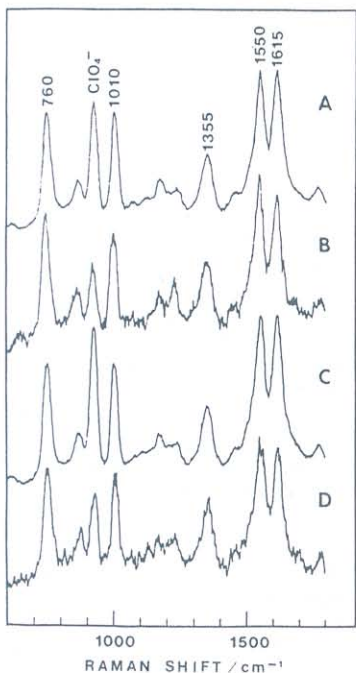


Fig. 1. (left) The pulse energy dependence of UV resonance Raman spectra of a monomer mixture (A and B) and dimer (C and D) excited at 225 nm.

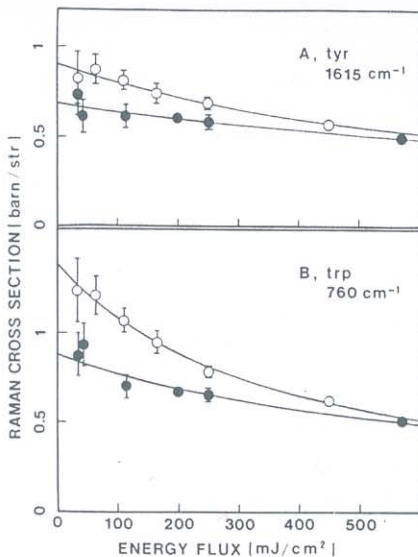


Fig. 2 (right) Dependence of Raman intensities of tyr and trp residues upon excitation pulse energy. Monomer saturation (O) dimer saturation (●).