electronic transition in molecules. Two-photon absorption occurs by a non-linear process in which two photons simultaneously interact with a molecule to induce a transition. The energy of the resultant excited state equals the sum of the energies of the incident photons. What is actually measured in such an experiment is the excited fluorescence. Three main requirements should however be fulfilled for a two-photon absorption-excited fluorescence experiment to be successfully performed. First the excitation frequency must be chosen to equal one half that of an allowed transition. Second, the sample must not absorb the excitation frequency by a one-photon process and, third, a reasonable fraction of the resultant fluorescence should occur in transparent regions of the sample. Highlights of the method are: bulk excitation is achieved for solutions prepared with optically dense solvents, one-photon forbidden electric dipole transitions of even parity, *e.g.*, g → g or u → u, can be excited; the experimental set up can be easily assembled.

This note reports on the two-photon spectra of 2,2′-bipyridine in solutions of organic non-polar solvents and in the solid state. Excitation was obtained by a nitrogen pumped dye laser with tuning ranges 5200–5900 Å, (cumarine 153), and 5650–6100 Å, (rhodamine 6G). The excited fluorescence, isolated by a filter with a 3300–4000 Å transparent window, was detected with a 1P28 photomultiplier tube. The anode current was fed to a Box-car averager for signal processing. The spectra were registered continuously by scanning the spectrum of the dye with a step motor driven holographic grating. The excited fluorescence was check to ensure a quadratic dependence on laser power. The normalized spectra of different solutions (cyclohexane, carbon tetrachloride, benzene, almost saturated) do not differ noticeably. Bands at 33613, 34246 and 34662 cm^{-1} were detected, whose assignment seems correctly be cast as ^1A_g (O–O), O–O + 615 (a_g) cm^{-1} and O–O + 995 (a_g) cm^{-1}, respectively. The 615 and 995 cm^{-1} lines are Raman active vibrational modes of trans 2,2′-bipyridine [1]. The calculated value of the ^1A_g (O–O) transition is 35945 cm^{-1} [2].

The assignment of other features, *e.g.*, 35087, 36036 and 36670 cm^{-1} seems ambiguous being, possibly, either ^1B_u (O–O) (magnetic dipole transition), ^1B_u + 1000 (b_u) cm^{-1}, ^1B_u + 1500 (b_u) cm^{-1} or ^1A_g + 1500 (a_g) cm^{-1}, ^1A_g + overtone$_1$, ^1A_g + overtone$_2$, respectively. In fact, a ^1B_u transition has been calculated at 34977 cm^{-1} [2]. All the frequencies were observed in solution and in the crystal without major differences. The decay time for fluorescence both in solution and in the crystal is of the order of few nanoseconds which would indicate a strong interaction with the environment, either solvent molecules or other molecules in the crystal.

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High and Low Affinity Ca^{++} Binding Sites in Troponin-C Studied by Synchrotron Radiation

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We have studied the structure of calcium binding-sites of Troponin-C using synchrotron radiation at the Frascati storage ring 'Adone'. We have measured the X-ray absorption spectra of the protein. From the analysis of the X-ray Absorption Near Edge Structures (XANES) [1] we have found direct evidence of different local structures for the low and high affinity Ca^{++} sites.

Skeletal muscle Troponin-C (TNC) is one of the three subunits of Troponin which provides Ca^{++} sensitivity to myosin ATPase: this occurs only when Ca^{++}, released from sarcoplasmic reticulum, is bound to TNC. TNC belongs to a family of homologous calcium-binding proteins, which includes, among others, parvalbumins and the ubiquitous calcium-dependent regulator [2]. A common structural feature of these proteins is the presence of a number of specific Ca^{++}-binding domains referred to as EF hands [2]. EF hands were observed first in the crystal structure of carp parvalbumin and, thereafter, the presence of four EF hand structure has been predicted in TCN on the basis of the known amino-acid sequence [2]. The coordination of Ca^{++} in every EF hand so far studied can be represented by an octahedron [3]. TNC has two classes of Ca^{++}-binding sites; the first class comprises two high affinity sites for Ca^{++} (Kd = 2 X 10^{-7} *M*) which are not fully specific

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since they also bind Mg^{++}, though with a much lower affinity (Kd = 10^{-4} *M*); the second class comprises two sites specific for Ca^{++} with a Kd of 2 X 10^{-5} *M* [4,5].

Two preparations were obtained by lyophilizing: first, 0.2 m*M* TNC in 0.38 m*M* $CaCl_2$; second, 0.2 m*M* TNC in 0.7 m*M* $CaCl_2$ and 2 m*M* $MgCl_2$.

In the first preparation calcium is bound only at the high affinity sites, while in the second both the high and low affinity sites for Ca^{++} should be occupied.

Our results show that the XANES of the two high affinity sites of TNC in the absence of Mg^{++} are very similar to those found in parvalbumin [2], suggesting an octahedral symmetry typical of the EF hand. The XANES of TNC shows different features under more physiological conditions, *i.e.* in presence of Mg^{++} and with all the four Ca^{++} sites occupied. The XANES structures are then broader probably because two different types of sites are occupied by Ca^{++}: the Mg-modified high affinity sites and the low affinity sites. The XANES of the latter sites are clearly different both for a chemical shift of the transitions to t_{1u} final states of about 0.4 eV toward lower energies and for a different splitting of the structures. The different features of XANES should be associated with a different Ca–O charge transfer, a variation of the Ca–O distance and a distortion of octahedral symmetry.

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Ultrasonic Propagation in Highly Concentrated Bile Salts Aqueous Solutions

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To investigate the solute–solvent interaction between bile salts and water molecules [1,2] ultrasonic absorption and velocity of aqueous solutions of sodium deoxycholate have been measured at 20 °C as a function of frequency (5–300 MHz) and concentration. Experimental results are in agreement with previous literature data [3]. Measurements of density

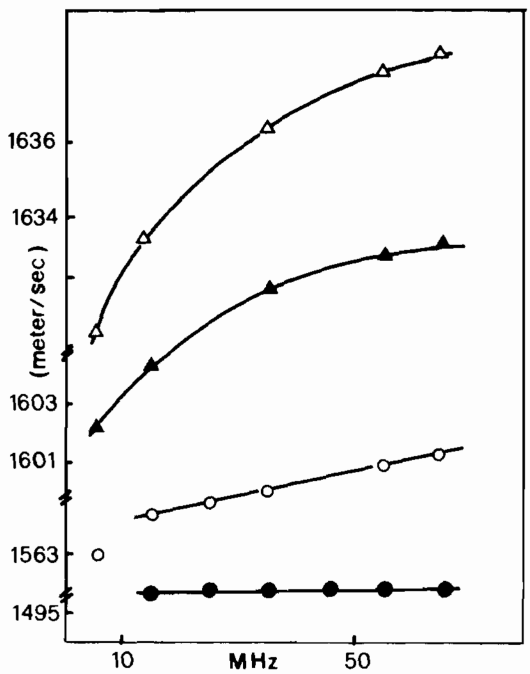

Fig. 1. Sound velocity *vs.* frequency at c = 0.08, ●; c = 0.34, ○; c = 0.47, ▲; and c = 0.72, △, mol/l.

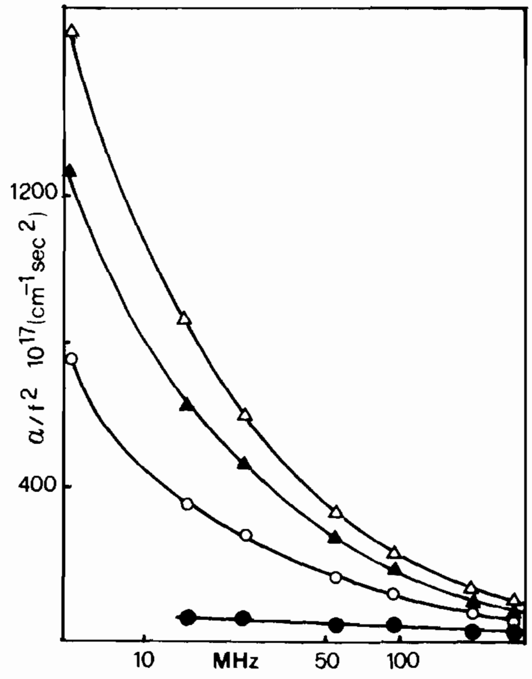

Fig. 2. Ultrasonic absorption *vs.* frequency at c = 0.08, ●; c = 0.34, ○; c = 0.47, ▲; and c = 0.72, △, mol/l.