

Reports

High-Pressure Ruby and Diamond Fluorescence: Observations at 0.21 to 0.55 Terapascal

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A diamond-anvil, high-pressure apparatus was used to extend the upper pressure limit of static laboratory experiments. Shifts of the R_1 strong fluorescent line of ruby were observed that correspond to static pressures of 0.21 to 0.55 terapascal (2.1 to 5.5 megabars) at 25°C. Sensitive spectroscopic techniques in the pressure range 0.15 to 0.28 terapascal were used to observe ruby and diamond fluorescence separately; these two fluorescent emissions overlap strongly at high pressures. At pressures greater than approximately 0.28 terapascal, the diamond fluorescence diminished and the ruby fluorescence reappeared strongly. Pressure was determined by extrapolation of the calibrated shift of the ruby R_1 line.

STATIC HIGH-PRESSURE EXPERIMENTS in the recent past have made possible the first measurements of the properties of solids at pressures above 0.1 TPa (1). The materials studied include metals, oxides, and several materials of interest in solid-state physics (especially hydrogen) and geophysics. Techniques need to be developed for observing insulator-to-metal transitions and for producing high-temperature superconductors at pressures above 0.3 TPa (2). Studies of materials at such pressures are also of importance in geophysics because of interest in attaining experimental data at conditions corresponding to those in the earth's interior (up to 0.35 TPa). In this report we describe the results of experiments

in which the range of static pressure generation has been extended to 0.55 TPa.

It is essential in these experiments to calibrate the pressure with an internal standard (3). The highest calibrated static pressure reported previously by this laboratory was 0.18 TPa (4). At that time it was possible to achieve higher pressures (0.25 to 0.3 TPa), but the maximum values were determined indirectly from the external load (5). At these higher pressures, experimental measurements in the diamond apparatus have been hampered by anomalous fluorescence effects that were thought to result from changes in the ruby crystals that are used as internal standards. In the experiments reported here, we determined that the

spurious emission at pressures above 0.1 TPa arises in the diamond anvils of the apparatus and not from ruby. The two fluorescent emissions, from the ruby internal standard and from the diamond, were observed separately in this study, and the maximum pressure of 0.55 TPa was determined from the ruby pressure-calibration scale.

The diamond-anvil, high-pressure apparatus (5) that we used was modified to include a port for angular incidence of the laser beam, which generates fluorescence in ruby crystals located in the sample chamber. The sample chamber contained, in addition to ruby crystals, either gold or tungsten, which served as a pressure-transmitting medium. Figure 1 shows diagrammatic sketches of the various laser beam configurations in the diamond cell. The laser beam (argon ion or helium-cadmium laser) for excitation of fluorescence and the Raman effect was introduced either in epi-illumination (vertical incidence and vertical emission) as in the earlier study (5) or at 45° from the surface normal to the upper diamond anvil. Figure 1A shows the epi-illumination configuration in which the fluorescent emission of ruby crystals in the sample chamber is coincident with diamond fluorescence along the same path.

The configuration for angular incidence is shown in Fig. 1, B and C. The incident laser beam converges to a spot 2 to 5 μm in diameter, and fluorescence originating in the diamond is isolated by separation of the paths and by optical spatial filtering. The emission path excludes most of the diamond fluorescence, and thus ruby fluorescence in the sample can be detected. One can observe the diamond fluorescence free of ruby emission by focusing the laser beam at points inside the diamond itself (Fig. 1C); a strong increase in the fluorescence intensity could be observed as the focal spot was moved vertically away from the sample into the interior of the diamond. The two sources of fluorescence could be separated by use of this configuration. The fluorescence and Raman spectra were measured with a non-scanning, triple-grating, 0.6-m spectrometer (Spex Industry, Triplemate). An intensified diode array (Princeton Instruments, optical spectrum multichannel analyzer) was used. This system was more sensitive by about three orders of magnitude than the apparatus used earlier, a 0.5-m scanning spectrometer (5).

Pressure generation in the present study was achieved with beveled diamond anvils

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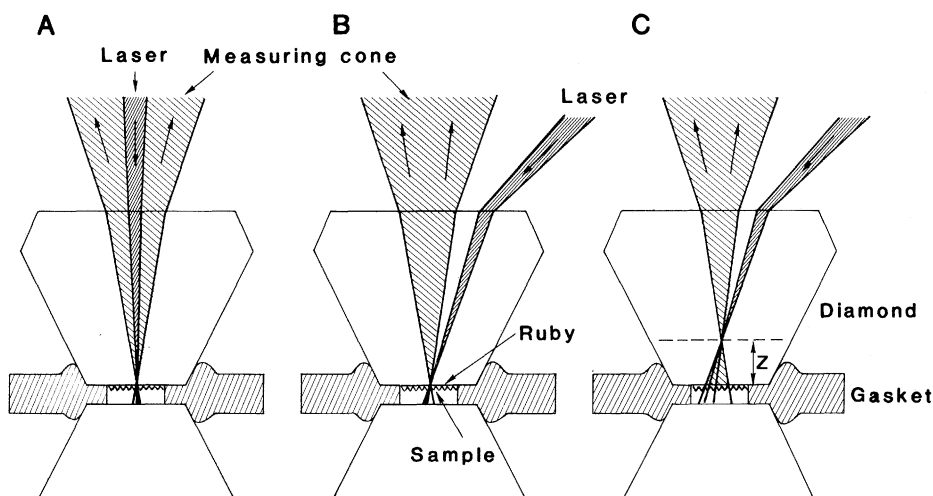


Fig. 1. Sketches of the laser beam geometry for fluorescence measurements of diamond and ruby in the diamond-window, high-pressure cell. (A) Configuration with vertical incidence. The beam is focused on a ruby grain in the sample chamber. The fluorescence emission is coincident with the laser beam. The fluorescence of the entire diamond as well as the ruby in the sample chamber is detected. (B) The laser beam is incident at an angle of 45°, focused on a ruby crystal in the sample chamber. The effects of interference from the diamond are minimized. (C) The laser beam is focused at an interior point in the diamond to measure fluorescence as a function of the vertical distance from the high-pressure face (Z).

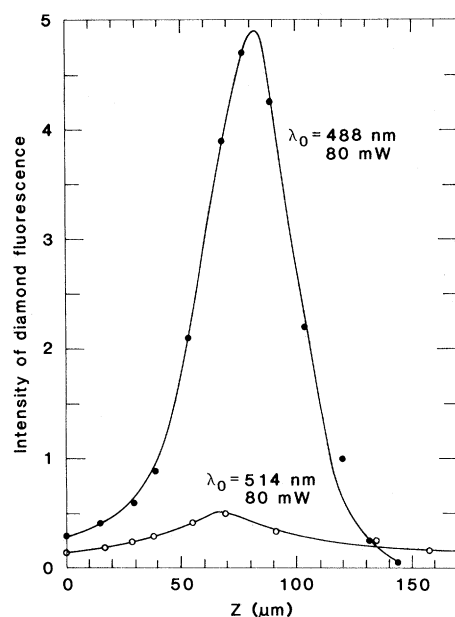


Fig. 2. Plot of the intensity of fluorescence of diamond as a function of Z . Pressure decreases vertically along Z ; the fluorescence increases to a maximum and then decreases. The pressure falls from 0.21 TPa at $Z = 0$ to 0.1 TPa at $Z = 130$ μm , on the basis of the observed shifts of the diamond Raman line (7). The measurements are shown at laser excitation wavelengths of $\lambda = 488$ nm and 514 nm, both at 80 mW. The intensity is an integrated measurement over the wavelength range 499.2 to 547.5 nm.

similar in most respects to those described by Bell and his associates (5). The diamond anvils that were selected had a high nitrogen content, which ensured high strength. The bevel that was ground to a relatively high angle (5) to the usual culet and flat pressure surface produced a steep pressure gradient, with the region of highest pressure located in the central portion of the sample. The highest pressure region in the sample chamber had a diameter of approximately 5 μm . The sample pressure was referenced against the ruby gauge calibration of Bell *et al.* (4), based on the gold internal standard (6). This scale, whose primary calibration extends to 0.18 TPa, is a simple extrapolation of the function

$$P \text{ (TPa)} = 0.3808 [(\lambda_p/\lambda_0)^5 - 1] \quad (1)$$

where λ_p and λ_0 are the wavelength of the ruby R_1 line at pressure P and at 1 bar, respectively.

The intensity of the combined fluorescence of ruby and diamond is not completely reproducible from one experiment to the next or even at different pressures in the same experiment, unless a specific ruby crystal and a specific series of points in a given diamond can be observed. The intensity of ruby fluorescence in the sample chamber varies from crystal to crystal, apparently depending on such factors as grain size,

orientation, and state of stress. The intensity also decreases as a function of pressure and, thus ruby fluorescence is particularly difficult to resolve when there is overlap with the diamond fluorescence, the intensity of which increases greatly with pressure. The intensity of the diamond fluorescence apparently is stress-induced; it is first observed at approximately 0.10 TPa and then essentially disappears at pressures of approximately 0.28 TPa, depending on the particular diamond, the frequency of excitation, and the window of detection. The strong diamond fluorescence spectra observed at wavelengths between 451 and 850 nm was featureless, exhibiting no well-defined maxima or peaks. Thus comparison with relatively weak diamond fluorescence observed at 1 atm was not feasible. The intensity of the pressure-enhanced fluorescence is greater by more than two orders of magnitude than the 1-atm fluorescence of an average type 1 diamond with high nitrogen content. Thus the relationship between diamond fluorescence at high pressure and at 1 atm is unclear.

Figure 2 shows a typical plot of the intensity of the anomalous diamond fluorescence as a function of the distance above the high-pressure face of the upper diamond anvil, Z (Fig. 1B). We could determine the approximate pressure inside the diamond anvils by observing the frequency shift of the diamond Raman line (7). At $Z = 0$, the pressure is 0.21 TPa. As Z increased into the interior of the diamond, the pressure decreased. The fluorescence intensity increased to a maximum at approximately 0.18 TPa, then decreased, and finally diminished as the pressure in the diamond apparently decreased to less than approximately 0.1 TPa (at $Z = 130$ μm). The strong fluorescence emission occurred between approximately 0.11 and 0.21 TPa in this example. The two plots in Fig. 2 show the diamond fluorescence excited at two laser wavelengths, 488 nm and 514 nm, at the same power level (80 mW). Both the diamond and the ruby fluorescence intensity decreased with longer excitation wavelength; thus the ratio of the ruby signal to background does not improve if the 514-nm excitation is used instead of the 488-nm excitation.

Because ruby fluorescence decreases steadily with pressure even under the most favorable experimental conditions, the ruby R_1 line was obscured at least partially by the diamond fluorescence at pressures between 0.15 and 0.28 TPa. At pressures above 0.28 TPa on the ruby scale, however, the ruby lines reappeared strongly and could be moderately well resolved. A series of observed spectra showing the ruby R line is shown in Fig. 3. In these experiments the quality,

resolution, and intensity of the ruby lines varied with the individual grain or grains being observed and with the degree of optimization of the laser, spectrometer, and optical imaging system. The spectra shown are typical of ruby grains located in the central area (5 μm in diameter) in the sample chamber. The spectral shifts correspond to pressures of 0.21 to 0.55 TPa, based on extrapolation of the ruby scale as a continuous function (Eq. 1) (Fig. 3).

These experiments demonstrate that the difficulties encountered in measuring the fluorescence of ruby in the diamond-anvil, high-pressure cell at pressures above 0.15 TPa result from fluorescence originating in the diamond anvils. The overlap of the ruby and diamond fluorescence can be reduced if one uses a configuration that allows angular

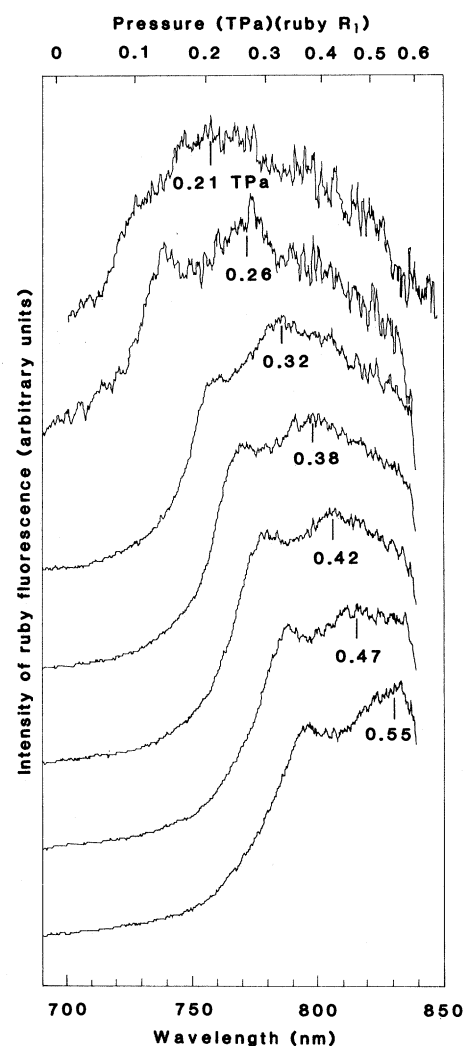


Fig. 3. Plots of the strong R line fluorescence emission of ruby crystals held at pressure in the diamond-window, high-pressure cell as a function of wavelength at several pressures from 0.21 to 0.55 TPa, based on extrapolation of the ruby scale (Eq. 1). The strong rise and maximum constitute the R line, the R_1 - R_2 doublet being resolvable at high pressures above the region of strong overlap with diamond fluorescence.

incidence of the exciting laser beam, with convergent focusing optics and spatial filtering and diode-array detection techniques. The ruby pressure scale can now be extended above 0.21 TPa because the fluorescence of diamond is diminished at higher pressures. The ruby fluorescence frequency shifts, measured at several points of increasing pressure in these experiments, correspond to pressures of 0.28 to 0.55 TPa on the extrapolated scale.

The disappearance of diamond fluorescence interference at pressures above 0.28 TPa is important in high-pressure experimentation because of the need to track the ruby fluorescence lines as they shift with pressure and to monitor the pressure above approximately 0.28 TPa. In the absence of strong diamond fluorescence interference,

measurements of weak scattering of light, such as Raman or Brillouin spectra of solids (2), are also feasible to pressures of 0.55 TPa. Such spectroscopic measurements should yield quantitative information about the insulator-metal transition in hydrogen and other condensed matter at high compression. The results would be directly applicable to the understanding of the deep interiors of the earth and other planets.

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8. Supported under Department of Energy grant DEAS0580ER10754, National Aeronautics and Space Administration grant NAGW214, and National Science Foundation grants EAR8418706 and EAR83114064. H. S. Yoder, Jr., and J. Ebert of the Carnegie Institution of Washington provided special funding support for construction of the diode-array spectrometer, for which we are extremely grateful. We thank R. J. Hemley for reviewing the manuscript and making useful suggestions.

19 February 1986; accepted 8 April 1986

The Human Immune Response to the OKT3 Monoclonal Antibody is Oligoclonal

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The availability of highly specific and homogeneous antibodies to human T cells by the hybridoma technique has elicited new interest in the clinical use of antibodies to lymphocytes as immunosuppressive agents. OKT3 is the murine monoclonal antibody that has been the most widely used in clinical transplantation to induce immunosuppression. This antibody recognizes a membrane molecular complex, exclusively present on mature human T lymphocytes, which is tightly linked to the T-cell antigen receptor. The long-term therapeutic use of murine monoclonal antibodies in vivo is hampered by the intense antibody response that occurs in most human patients. Thus, when administered alone, OKT3 manifests its immunosuppressive activity only during the 10 to 15 days that precede the onset of sensitization. The results presented here show, by use of isoelectrofocusing, that the antibody response to OKT3, already reported to be restricted in its specificity (only anti-isotypic and anti-idiotypic antibodies are produced), is in addition oligoclonal. This restriction of the anti-monoclonal response may suggest that an efficient way to circumvent the sensitization problem would be to administer consecutively different monoclonal antibodies presenting the same specificity but distinct idiotypes.

MONOCLONAL ANTIBODIES HAVE been used successfully in vitro as highly specific probes. As therapeutic agents, however, they have not proved so successful (1-6). In studies of their antitumor or immunosuppressive effects in vivo, their ability to deplete the target cells (tumor cells or normal T cells) gave rise to an initial enthusiasm, but it was soon realized that there were two major obstacles to be circumvented before monoclonal antibodies could be used on a long-term basis. The first obstacle, antigenic modulation, may result in annihilation of the antibody therapeutic effect, when the aim of therapy is cell depletion and not merely the removal from the cell membrane

of a functionally important molecule (2, 4, 7). The second obstacle, monoclonal antibody sensitization, occurs in most patients treated with the same monoclonal antibody for several consecutive days (4, 6-9). This sensitization is often intense and rapid and may abrogate the therapeutic effect of the monoclonal antibody and expose the patient to the risk of serum sickness. The results reported here suggest that the immune response to xenogeneic monoclonal antibodies is oligoclonal and for the most part specific for the antibody injected. This is interesting from the theoretical point of view since it illustrates in humans the notion that immunization with low doses of xenogeneic immunoglobulins administered intra-

venously induces a restricted immune response. It is also important clinically because it suggests a practical means of circumventing the sensitization side effect by shifting to another antibody with different characteristics.

Serum samples were collected from five renal allograft recipients treated prophylactically, that is, to prevent rejection, with the OKT3 monoclonal antibody (Ortho). As described elsewhere (10), these patients were participating in a randomized trial that included a total of 26 allograft recipients receiving OKT3 (5 mg/day injected intravenously for 13 to 30 days) either alone (six patients) or with azathioprine (3 mg per kilogram of body weight per day) and prednisone (0.25 mg/kg per day) therapy. The five patients tested showed intense antibody response against OKT3. In addition, the sera from four rhesus monkeys that received a skin allograft and that were prophylactically treated with OKT4 (Ortho) monoclonal antibody (5 mg/day for 10 to 16 days) (11) were also analyzed.

Analytical isoelectrofocusing was performed with thin-layer (0.5 mm) polyacrylamide gels (acrylamide, 5 percent weight to volume; bisacrylamide, 0.15 percent weight to volume; ammonium persulfate, 0.07 percent weight to volume), containing 0.08 percent (weight to volume) ampholytes pH 3.5 to 10 (LKB). The sera (10 μ l diluted by 50 percent in saline) were loaded onto sample applicators and focused at a constant

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