1. (Total 20%) The UV/Vis experiment:
Lysozyme (AMRESCO, USA), concentration 180-200 µM, was dissolved in double-distilled deionized water (pH 4.4-4.5) or in phosphate buffer saline (PBS), pH=6.5. The sample preparation by both media followed by spectral analysis was estimated. The initial protein concentration was check with UV/VIS spectrometer (Jasco V-550) by the solution absorption at 280 nm. A molar absorbance of lysozyme at 280 nm \( (3.7547 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}) \) served to calibrate the protein concentration by the measured absorbance at the Soret band maximum. The concentration of lysozyme in solution was measured before adsorption; then carboxylated nanodiamond, concentration 4-10 mg/ml, was added to the solution. To ensure maximum adsorption, the protein solution and the diamond powder were thoroughly mixed using a shaker for 2 h, after that the mixture was centrifuged and washed several times with deionized water. After first separation of nanodiamond with adsorbed protein, the residual concentration of protein in supernatant was measured. The quantity of protein adsorbed by nanodiamond was estimated by the difference between initial and residual protein concentrations. In Figure 1 on the right, typical UV-VIS spectra are shown for lysozyme in water and PBS before and after interaction with nanodiamonds. The spectra measured after diamond addition and thorough mixing show quite dramatic removal of the proteins from the solution as a result of physical adsorption of protein by nanodiamond surface. For 5 nm nanodiamond the adsorption is extremely effective as indicated in the spectra in the above figure. The protein-nanodiamond interaction, determining the adsorption, depends on the environment as well as on nanodiamond surface properties. Saturated adsorption of lysozyme is observed for 5 nm nanodiamond in PBS. We estimate that 1 mg of 100 nm nanodiamond adsorbs on their surfaces up to 45 µg of lysozyme from water solution and 80 µg from PBS; 1 mg of 5 nm nanodiamond adsorbs up to 200 µg of lysozyme from water solution and 500 µg from PBS. Note also, negligible absorption at 280 nm was observed after second washing of supernatant fraction of the sample; it means that adsorption is strong and stable.

In the above underlined part, we can calculate the total absorbed lysozyme base on the provided UV/Vis spectra in Figure 1. Explain why? You don’t need to be quantitative, but rather qualitative answer is sufficient. (20%)
2. **(Total 30%)** IR spectra: Refer to Figure 2. Infrared spectra of carboxylated/oxidized nanodiamonds are plotted in Figure 2. The acid treatment of nanodiamond creates the functional groups on the diamond surface can be observed using FTIR spectroscopy. Figure 2(a) and (b) are from the same carboxylated 100nm diamonds, except Fig. 1(a) was measured in air with higher diamonds concentration (20mg/60µl) while (b) was taken in vacuum chamber at lower concentration (1.4mg/60µl). The band near 1630 cm\(^{-1}\) needed to be assigned, which arise between neighboring carboxylated nanoparticles at high concentration. The assignment is evidenced from the disappearing of this band when the spectrum was taken in the vacuum (~10\(^{-6}\) torr) as shown in Figure 2(b). For smaller 5nm diamonds, this band shifted to higher wavenumbers presumably arises from the intermolecular interaction of the neighboring bonding due to small particle size.

(a) What is the band A in the figure? Why it disappears in (b)? (10%)
(b) What are the bands B, C, D, E? (20%)

3. **(Total 40%)** Fourier transform interferometer: In Figure 3, D is a fixed mirror, E is a moving mirror. A monochromatic light of intensity \(I_0\) and frequency \(\nu_1\) incident into a Fourier transform Interferometer. If the electric field of the light going through the fixed and moving mirrors are

\[
E_1(\nu_1) = \frac{E_0}{\sqrt{4}} e^{i2\pi\nu_1 z} \quad \text{and} \\
E_1(\nu_1) = \frac{E_0}{\sqrt{4}} e^{i2\pi\nu_1(z+\delta)} , \quad \text{Respectively, where}
\]

\(\delta\) is the optical path difference of the two electric fields undergoes different paths.

(a) Derive the observed light intensity \(I(\delta)\) at the detector G position as a function of \(\delta\). (20%)
(b) Plot \(I(\delta)\). (5%)
(c) Plot \(I(\delta)\), if the incident light has two frequency, say \(\nu_1\) and \(\nu_2\). (5%)
(d) Plot \(I(\delta)\), if the incident light has a broad frequency distribution. (10%)

4. **(Total 10% for this problem)** Raman spectroscopy: What is Raman the origin of Raman spectrum, i.e. what is Raman shift? (10%)