Table 2| Troubleshooting table

|  |  |  |
| --- | --- | --- |
| Step | Problem | Solution |
| 26 | There are air bubbles in the constituted sample | The lyophilized protein must be suspended gently to avoid the generation of bubbles in the sample. |
|  | There are air bubbles in the cell  The operation is not completed in the initial delay time. | The CaF2 window is not clean or the volume of sample is not enough. Demount the cell and clean it very gently. More sample is needed for the experiment.  Prepare another sample and repeat the experiment. |
| 27 | There is a very low IR absorbance signal or no signal | Check the cell spacer and ensure it is>30μm.Check the protein concentration and ensure it is high enough. |
| 31 | Sample in the cell leaks before completion of the experiment | The entrance hole and exit hole must be sealed well. Check the cell spacer and ensure it is <80 μm. |
| 42 | ω is not nearly 2/3 or there is great variability between the value of ω obtained from the three independently acquired spectra. | Check the cell spacer and ensure the spectrum for protein in H2O is obtained in very thin films (<10μm). Alternatively, ω can be obtained from the spectra of very thin films of H2O[63](#_ENREF_63). By such means, ω has been found to be very nearly constant for proteins. If ω is pH- and/or temperature-independent, the value of ω will shift under different pH or temperature. Control the experimental conditions and ensure they are consistent during the experiment. |
| 44 | F of the last point >5% | The spectra should be recorded for a longer time. |
| 45 | F versus exchange time plots cannot be fitted well with Eq.(3) | Check if AII∞ is correct. Incorrect AII∞ values will affect the plots. Random errors arise through dissolution of Ca2+ from the windows into the solution, through the elevation of temperature when the thin film of solution resides in the intense infrared beam, and through baseline drift. This will affect the data. Try to increase the protein concentration and ensure that there are no cosolvents in the sample. All can be corrected by careful and ingenious experimental techniques. |