# The PDP 2005 Annual Progress Report

## Infinite Quanta, Inc.

**A Non-Profit 501(c)(3) Scientific Research Organization**

2005 has been a year of progress in every respect with scientific and technical advancements and corporate contributions. Highlights this year included $40,000 equipment contributions from 3M, Tektronix, and Millipore Corporations. Their contributions are appreciated and will ensure continued progress and achievement in 2006. Conversations with the contributors have brought forth new technical insights and methods for doing good science. It is hoped that other corporations will realize our deep commitment and intentions, as well as the technical and scientific potential, of the Peptide Dynamics Project (PDP), and might contribute the best equipment and expertise they have to offer.

Due to contributions from Ametek Signal Recovery, National Instruments, and Mettler-Toledo, the Phase I Dielectric Spectrometer (P1DS) was built and tested in 2004. In 2005, many spectra were run on simple protein samples at ambient temperature and pressure. The P1DS was the first genuine attempt to study the complex dielectric spectra of peptides and proteins. Throughout the year, many lessons were learned and prudence dictates that upgrades are required in the areas of electronic, sample cell design, chemical techniques, sample preparation, environmental control, and software. Please visit our below website for the details of suggested improvements for the proposed Phase II to V Dielectric Spectrometers (P2DS to P5DS).

It is important to consider that the objective of observing the dipoles of the intramolecular structural motifs in peptides and proteins will require constant refinement in all areas of instrumentation, sample preparation, and environmental control. The primary reason is that aqueous solutions have a rather large dielectric constant, ~80, and it is estimated that the dielectric constants of the intramolecular structural motifs will be on the order of 3 to 20. This is a classic instrumentation scenario of observing and studying a small signal cloaked in a large signal, that of the motifs within water, respectively. However, with the advances of the past two decades in electronics, software, and computers, observing these intramolecular motifs has the greatest potential then anytime in history. That is why the PDP has good timing and the greatest potential of seeing this phenomena.

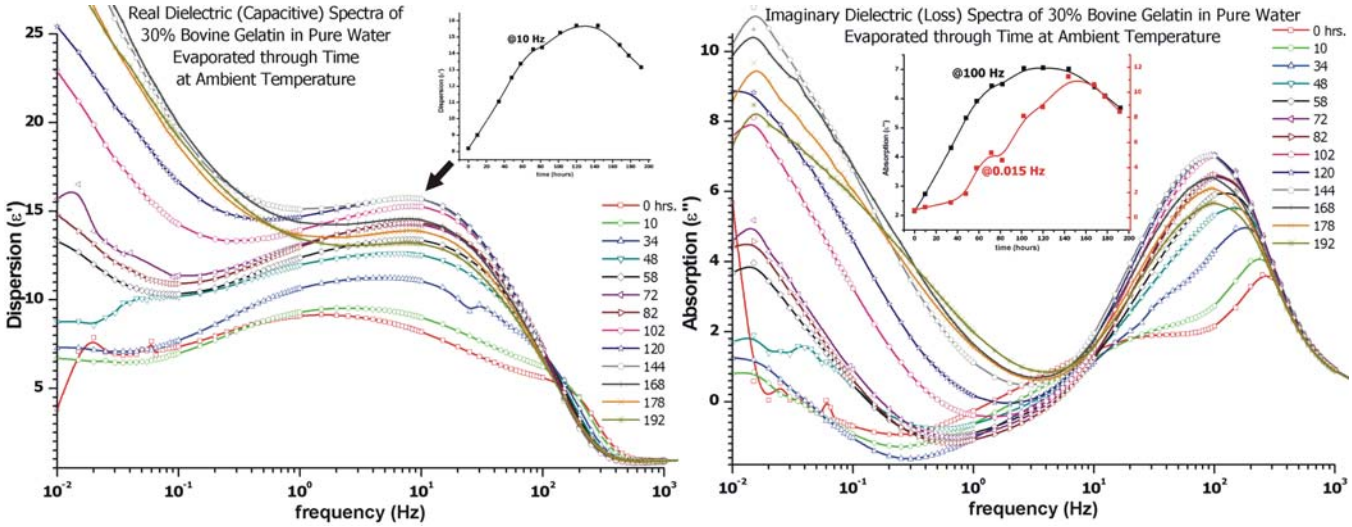
Sample preparation is critical to reducing undesired effects while enhancing desired effects. All previous dielectric studies, by other groups, were done with pure peptides and proteins in aqueous solution, allowing the macromolecules to freely rotate within solution. An early idea of the PDP, is to enclose the peptides and proteins in a matrix so as to impede all rotational motions of the entire molecules. Initially, it was considered to super-cool, but not freeze, the biomolecules in the solution. This would create a highly viscous medium and greatly impede molecular rotations. It would also reduce the Maxwell-Boltzmann distribution, thereby reducing the noise created by the much smaller conductive water molecules. This technique is potentially viable, however, highly precise solvent-based temperature baths and controllers, and sample cells, are required to perform these experiments. It is proposed within the 2007 budget to obtain this capability for the super-cooled experiments.

Another technique was proposed to impede the molecular rotations by encapsulating the peptides and proteins in a chemical matrix at room temperature. This would require semi-solid substances that possess a fairly low dielectric constant and do not chemically react with, or diminish the dielectric response of, the peptides and proteins under study. Such substances would be collagens, gelatins, agars,

and other electrophoretic gels. All of these substances are biologically derived and have the potential of creating a semi-solid chemical matrix suitable for studying peptides and proteins in solution.

Gelatin was first studied using the P1DS at room temperature. Gelatin is a protein derived from collagen. Both substances have a high proline and hydroxyproline content, roughly 20 to 50%. Proline is one of the twenty amino acids and the only one with a rigid ring structure. The α-helix requires a certain amount of flexibility of the amino acids and proline is known to break the helical structure because of its inflexible ring structure. Therefore, gelatin is thought to have a greatly reduced α-helical content and thus would lower the overall permanent dipole moment and dielectric constant as compared with proteins possessing all α-helices.

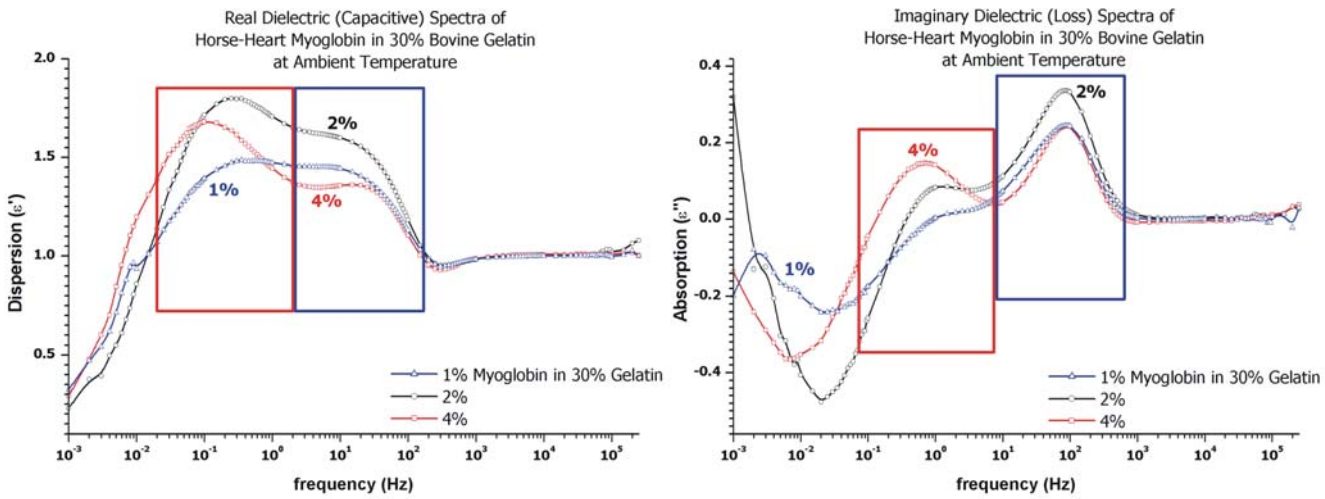
Many studies of gelatin were performed, however, they all culminated in a study in which a single sample of 30% bovine gelatin in pure water was allowed to evaporate over an eight day period. Dielectric spectra were obtained at regular intervals once or twice a day. The below plots show the capacitive and loss spectra, respectively, over the frequency range of 10 mHz to 1 kHz.




As can be seen, all of the spectra follow a similar path with the amplitudes changing over time as the water gradually evaporates from the sample. An additional inset plot was made at 10 Hz of the capacitive responses and two additional inset plots were made at 15 mHz and 100 Hz of the loss spectra. All three inset plots exhibit similar slopes and behavior, in that a maximum was reached at approximately the same time. These observations prove that water does play an important part in the dielectric spectra of gelatin, and perhaps proteins in general, and that an optimum protein concentration exists that enhances the complex dielectric properties of the sample. Subsequent studies proved that the optimum concentration was approximately 40 to 50% gelatin in water.

Myoglobin was also studied in the P1DS. Horse-heart myoglobin was dissolved in 30% bovine gelatin at 40°C, while the gelatin was semi-liquid. In this study, the sample is one of the three concentrations of myoglobin in 30% gelatin. The reference is the 30% gelatin in pure water. The complex admittance of the sample was then divided by the complex admittance of the reference, as outlined in Equation I14 of the Instrumentation and Experimental section of the PDP website.

The capacitive and loss spectra of 1, 2, and 4% myoglobin in 30% gelatin in pure water are as follows.



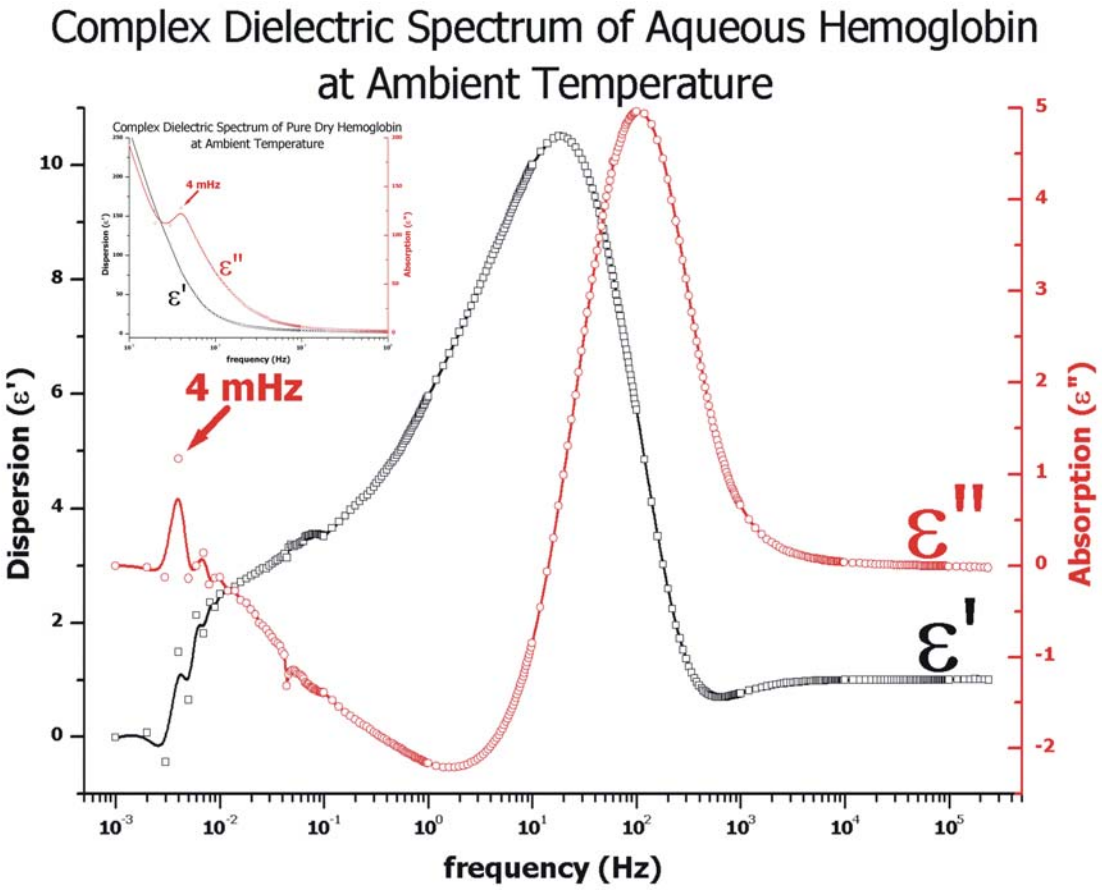

There are two interesting regions marked by a red and blue boxes. The blue box is probably water interacting with the electrodes, called electrode polarization, and is consistent with the responses around 10 Hz in the capacitive and 100 Hz of the loss spectra of the above pure gelatin in water studies. The response of the red box does not exist in the above pure gelatin studies and is probably that of myoglobin. The three concentrations of myoglobin were measured to ensure that the complex dielectric spectra did vary with only the concentration of myoglobin.

The absolute concentration responses are not directly correlative, probably because of varying scan times between the samples and thus water evaporating from the samples, as proven by the above gelatin studies. The relative concentration responses, however, are quite revealing. In the capacitive spectrum, notice a one-to-one relationship between the 1% myoglobin and water responses, shown in blue. The 2% myoglobin is roughly two-to-one, shown in black, and similarly the 4% myoglobin exhibits a four-to-one response, shown in red. The loss spectra show similar behavior, but with inverse ratios.

The final study was hemoglobin in the P1DS. 30% bovine hemoglobin was dissolved in pure water and studied at room temperature within the full frequency range of 1 mHz to 250 kHz. The complex dielectric spectra are as follows in which the capacitive spectrum is shown in black and the loss spectrum is shown in red. As can be seen, the strong response due to the electrode polarization with water is apparent at 10 Hz for the capacitive spectrum and 100 Hz for the loss spectrum.

It is clear that the electrode polarization effect must be reduced in the next year. The literature states that using Pt-Black coating on the electrodes reduces the effect by three to four magnitudes. It was realized a few months ago that the mechanism of the Pt-Black coatings is to create a thin layer of hydrogen gas between the electrodes and the sample. Hydrogen gas is a non-polar substance that would act as a conductive barrier between the sample and electrodes. It is proposed, therefore, that the next line of studies will be to simply see the effects of various petroleum-based coatings on the sample cell electrodes. It is hoped that these studies will provide a simple, inexpensive, and effective method of reducing the electrode polarization effects.

Ignoring the electrode polarization response, there is an intriguing response in the 4 to 20 mHz range. The rather strong peak at 4 mHz may be a resonance effect of hemoglobin, or a structural motif within hemoglobin. The lesser peaks centering around 8, 12, 16, and 20 mHz also indicate harmonic responses from the primary absorption of 4 mHz.




It must be emphasized that the 4 mHz response exhibits normal dispersion because the capacitive response is increasing with frequency, indicating a resonance effect. Usually, dielectric spectra are strictly observed as anomalous dispersion and the underlying mechanism involves relaxation processes.

To confirm the existence of the 4 mHz response, powder-dry bovine hemoglobin was compressed between the electrodes and the admittance was compared against that of air. The above inset shows a clear loss peak at 4 mHz. This confirms the 4 mHz response and its derivation solely from hemoglobin. It also indicates the importance of water in amplifying the primary and harmonic responses and the possibility of resonance, as opposed to relaxation, mechanistic processes.

In conclusion, the past year has proven to be a good journey. Some initial and promising scientific inroads were laid with the gelatin, myoglobin, and hemoglobin studies. Many technical lessons were learned and continued work will hopefully provide solutions to the many deficiencies of the P1DS's electronics, sample cell design, sample preparation, and environmental control. I believe the current results and lessons, leading to the new ideas, proposals, and aspirations, of the PDP in 2005 have proven a success and provide hope for 2006 and 2007.