Evaluation of Protein Hydration by Vapor Sorption Methods

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Statement of Purpose: Enzymes and proteins perform essential roles in the digestion, transport and processing of fats and oils. Enzymes are important for all organisms in the formation or hydrolysis of fats (lipids). Even viruses may contain genes encoding lipases. Considering that hydration is a major factor in the activity and selectivity of enzymes in organic solvents the water and organic vapor sorption isotherms would provide fundamental information on the mechanism of the hydration of enzymes and proteins.

Methods: The water vapor sorption behavior of untreated and protein treated drug samples were investigated by means of Dynamic gravimetric Vapor Sorption (DVS). Samples of an amorphous solid dispersion in a binder (sample PP) were treated to incorporate Protein (sample PPZ) into the amorphous system. Samples were kindly supplied by University College London (UK). Surface wettability experiments were performed by Inverse Gas Chromatography (IGC). Powder samples were packed in a column with 30 cm length and a 4 mm ID. Samples were run at a series of surface coverages with alkanes and polar probe molecules to determine the dispersive surface energy distribution as well as the acid-base surface energy distribution. Each column was pre-conditioned for 2 hours at 30°C and 0% RH with helium carrier gas to remove any physisorbed water.

Results: Water sorption results show moisture-induced crystallization for PP between 40 - 60% RH. This crystallization event is significantly reduced with the addition of protein, indicating that the protein acts as a stabilizer. For both untreated PP and treated PPZ powder samples, video microscope images were taken at the end of each RH step. The PP sample shows distinct change in the visible appearance of the sample at high RH conditions. At low water concentrations water molecules bound to specific water binding sites at the protein surface, but at higher water concentrations, >50% RH, the protein-bound water molecules must reach their limit as the isotherms indicate that condensation or over saturation occurs.

Figure 1 shows the wettability (specific surface energy divided by the total surface energy) profiles for the samples as a function of measurement relative humidity. The wettability of the treated PPZ sample increases with increasing relative humidity. At higher RH conditions, water vapor binds at the surface of enzyme, thus shielding these sites and leaving more hydrophobic sites exposed. In addition, Lewis acidity and basicity values would decrease with increasing %RH as water molecules progressively would cover the polar surface groups of the sample. These groups would be completely covered by water at 70% RH. There appears to be an optimum water concentration at which the substrate displaces water molecules to interact with the protein. The untreated sample doesn't show a change in the specific surface energy but the dispersive surface energy increases with the humidity, which may be explained by the irreversible phase change shown by the DVS results at above 50% RH. In the case of the treated sample the dispersive surface energy doesn't change significantly, but the specific surface energy (acid/base properties) changes with increasing RH up to 70% RH before decreasing at 80% RH. This supports the DVS results which showed water condensation on the surface of the material at above 70% RH.

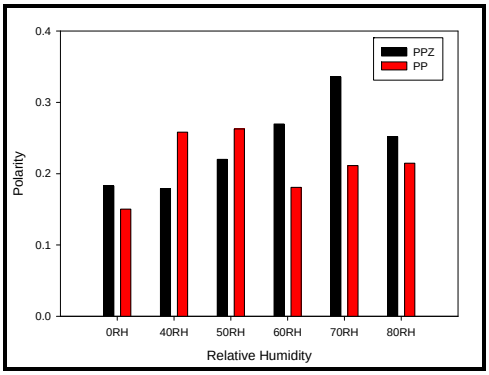

Figure 1. The wettability of the samples as a function of the relative humidity at 30 °C.

Conclusions: The DVS moisture sorption isotherms would provide a platform to study the interaction of protein charged or polar groups with water groups. The adsorption isotherms and surface characterization by IGC SEA would allow the determination of the water concentrations at which organic vapor would compete with water for adsorption on the protein.