ZETA POTENTIAL OF BOVINE SERUM ALBUMIN (BSA) PROTEIN

Zeta potential is a key physical parameter that describes surface charge on proteins. Although there has been interest in measuring the zeta potential of proteins for many years, the measurements are often difficult due to a variety of reasons including the "cooking" of proteins on the cell electrodes when using light scattering techniques. Electro acoustics provides an alternative approach for non-destructive measurement of protein zeta potential without sample preparation. This application note describes a study of investigating the zeta potential of bovine serum albumin vs. pH using electroacoustics.

Introduction

Bovine serum albumin (BSA) is a serum albumin protein that has numerous biochemical applications including immunoblots, immunohisto-chemistry, and use as a nutrient in cell and microbial culture. This protein does not affect other enzymes that do not need it for stabilization. BSA is also commonly used to determine the quantity of other proteins by comparing an unknown quantity of protein to known amounts of BSA. BSA is used because of its stability, lack of effect in many biochemical reactions, and low cost since large quantities of it can be readily purified from bovine blood. The basic physical properties of BSA are Molecular Weight = 69 kDa and the number of residues = 607.

Zeta (ζ) potential is the potential in the interfacial double layer (DL) at the location of the slipping plane versus a point in the bulk fluid away from the interface. In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle – see Figure 1.

The zeta potential of a protein can be used as an indicator of stability. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles (proteins) in a dispersion. According to general colloid chemistry principles, a dispersed system typically loses stability when the magnitude of the zeta potential decreases to less than approximately 30 mV (independent if the charge is positive or negative). As a result, there will be some region surrounding the condition of zero zeta potential (the isoelectric point, or IEP) for which the system is not particularly stable. Within this unstable region, the proteins may agglomerate, thereby increasing the measured size.

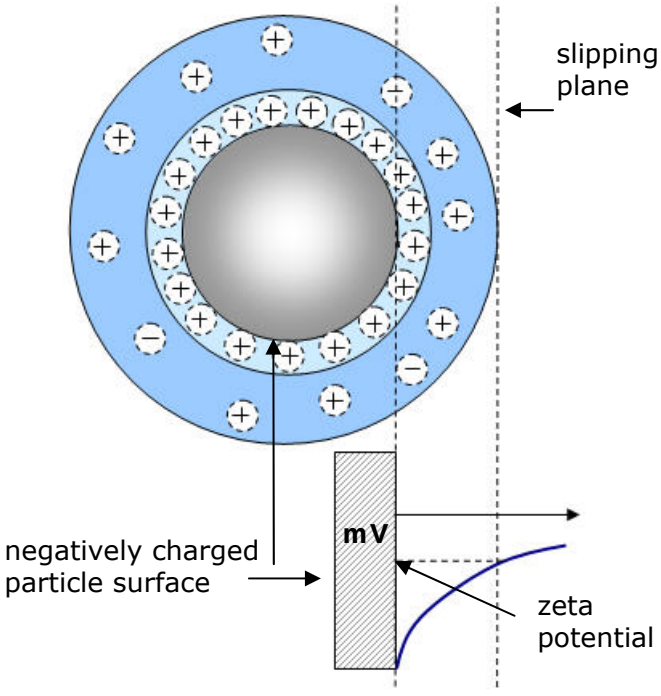

Figure 1

Zeta potential can be measured using various techniques including microelectrophoresis, electrophoretic light scattering, and electroacoustics. Light scattering techniques for measuring zeta potential using electrodes to apply an electric field can damage the

sample from Joule heating on the electrodes. This heating not only damages the sample but also degrades data quality since the induced electric field is changed by the protein "cooking" onto the electrodes.

The electroacoustic technique offers several advantages over light scattering including, most importantly, the ability to measure without this type of induced electric field. Such an electric field results in protein oxidation, destruction of the sample, and an unavoidable degradation of data accuracy. The sensor used for the electroacoustic measurements made in this study (see Figures 2 and 3) applies an acoustics wave to the sample rather than an electric field. The applied acoustic waves induce a dipole moment around the particles in front of the gold plated probe. The sensor measures the Colloid Vibration Current (CVI) created by the electric field from the dipole moments. This avoids the problems associated with light scattering techniques and can be used for protein samples containing at least 1 wt% of sample, or roughly 10 mg/ml.

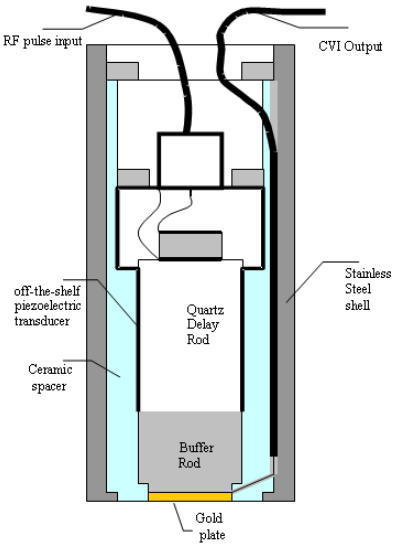

Figure 2: Cross-section of the electroacoustic probe

Figure 3: The DT-300 Zeta Potential Probe

Experimental

BSA has a natural tendency for aggregation under stress conditions (pH extremes, concentration, aging, higher ionic strength, fatty acid impurity, etc.). Protein interactions are strongly influenced by electrostatic forces. The charge density zeta potential at the slipping plane controls the strength of electrostatic interaction. For this test, the tendency for BSA to aggregate under changes in pH is investigated. A complete pH titration from pH 1.8 to 11.2 was conducted measuring the zeta potential at each stabilized pH value.

BSA lyophilized powder purchased from Fisher Scientific (CAS: 9048-46-8) was prepared by diluting in ultra-pure DI water. The samples were analyzed using the DT-300 zeta potential probe by adding drops of the protein suspension on top of the probe until the surface was covered (approximately 2 mL).
The pH titration was performed manually by starting with the native sample at pH 5.3, then changing pH using 0.1N HCl. Next a fresh sample was created and pH was changed using 0.1N NaOH. Five zeta potential measurements were made at each pH value and the average value was reported.

Results

Table 1 and Figure 4 show the results of zeta potential as a function of pH.

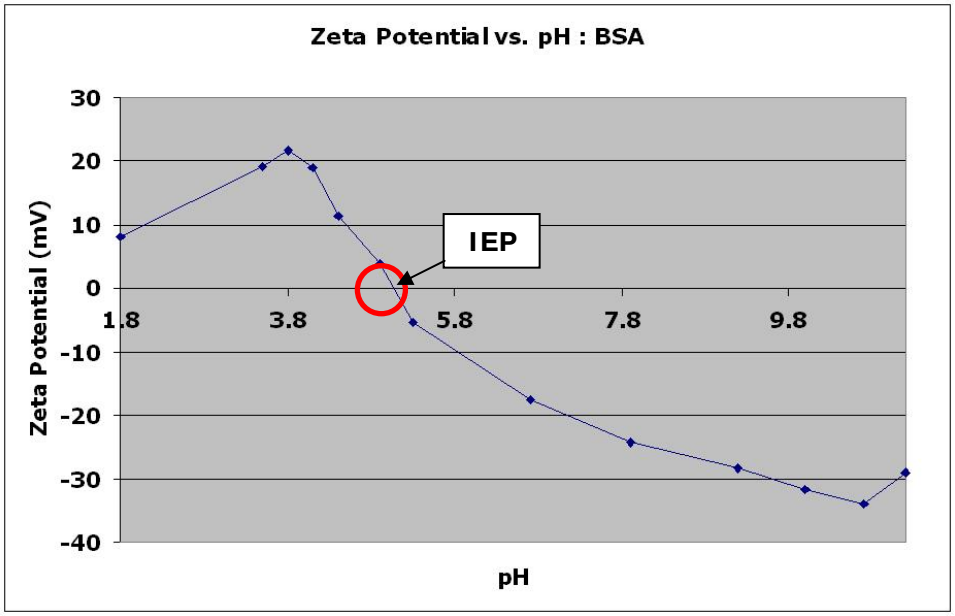

Figure 4: The relationship between pH and Zeta Potential for BSA

pH	CVI	Zeta	STD
1.8	157,701	8.2	0.17
3.5	365,881	19.1	0.24
3.8	415,370	21.6	0.26
4.1	363,881	19	0.08
4.4	218,582	11.4	0.07
4.9	72,347	3.8	0.62
5.3	104,411	-5.4	0.06
6.7	329,171	-17.6	0.11
7.9	453,600	-24.2	0.15
9.2	526,892	-28.2	0.15
10	593,132	-31.7	0.29
10.7	634,293	-33.9	0.4
11.2	541,855	-28.9	0.2

Table 1: The Colloid Vibration Current (CVI), Zeta Potential, and Standard Deviation values at various pH values for BSA

Conclusions

The zeta potential of the BSA sample varied as a function of pH as expected. Changes in the Zeta Potential for a protein imply:

- Conformational changes in protein structure
- Surface modifications
- Aggregation of macro-molecule
- Unfolding/denaturation of the protein

The zeta potential of BSA was easily measured using the DT-300 sensor. This approach to measuring zeta potential of a protein did not suffer from any of the challenges faced when using light scattering techniques.

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