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THE INVESTIGATION OF THE EFFECT OF HIGH CONCENTRATION AND PH ON MID-IR AND THZ SPECTRA OF BSA SOLUTIONS

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Key words: *high concentration, FTIR spectroscopy, THz spectroscopy, bovine serum albumin*

INTRODUCTION

Inside of a cell there is an enormous number of large molecules that transform the environment in a crowded one. The high concentration of these molecules cause important effects on the properties of the proteins [1-3].

It is already known that the pH value of the protein solutions strongly influences their conformation, stability and solubility. The pH value has a strong effect on the conformational changes of BSA, since pH changes are known to cause reversible conformational isomerization in albumin solutions [4-6]. Li S., et al (2007) makes a classification of the pH-dependent forms of albumin. These are: N are the normal or native forms, which are mostly found at neutral pH; B are the basic forms which occur above pH 8; F are the fast migrating forms which suddenly appear at pH values below 4.3; E are the expanded forms at pH below 3.5. All of these forms expose specific structure and functions while the conformational changes between different forms has a physiological importance [4,6].

Terahertz (THz) spectroscopy is an important tool for the study of protein structure and conformation. THz spectroscopy covers the spectral range from 3 cm^{-1} to 600 cm^{-1}, also known as the far-infrared (far-IR) region of the spectrum [7]

Terahertz time-domain spectroscopy (THz-TDS), provides a new method for study of structural changes and conformational flexibility of molecules by using collective vibrational modes in the terahertz frequency range of 0.1-3 THz. The protein motion is represented through the position of all normal modes of vibration that link the function of a protein to its structure [8].

Mid-Infrared spectroscopy probes molecular vibrations. Chemical bonds undergo various forms of vibrations such as stretching, twisting and rotating. With developments in FTIR instrumentation it is now possible to obtain high quality spectra from dilute protein solutions [9-12]. The most important advantage of Fourier Transform InfraRed (FTIR) spectroscopy for biological studies is that spectra of almost any biological material can be obtained in a wide variety of environments. Spectra of a protein can be obtained very well in single crystals or in aqueous solution. The chemical environment in which a peptide or protein exists influences its structure and stability. Modern FTIR spectrometers enable the study of very small quantities of biological samples, down to 10 mg. The size of the protein be analyzed using a FTIR spectrometer is not important. Another advantage is that there is no light scattering or fluorescent effects. Kinetic and time-resolved studies are also possible [13]. The spectrum obtained by FTIR is specific to the analyzed sample.

Bovine serum albumin (BSA) is the most abundant protein in cows' plasma (0.6 mM), and therefore the main component of colloid osmotic pressure. BSA is a multifunctional protein with an extraordinary ligand binding capacity. Its main functions involve the binding and transport of various metal ions, metabolites, nutrients and drugs [14]. It is a large (~66 kDa) protein that is negatively charged at the physiological pH. BSA is heart-shaped and comprise three helical domains (I, II and III) each comprising two subdomains (A and B) [15].

MATERIALS AND METHOD

Preparation of protein solutions.

We used BSA solutions at 8 pH values and at 12 protein concentrations in an universal buffer consisting of Na_2HPO_4 and citric acid.

The Albumin Fraction V was purchased from Carl Roth (≥98 %)and was used without further purification. All other chemicals including the Na_2HPO_4 and the citric acid were purchased from Sigma-Aldrich. Deionized water was used to prepare all the solutions. We prepared BSA solutions in universal McIlvaine buffer [17] at 8 pH values (2.2, 3, 4, 5, 6, 7, 7.4 and 8) and at 12 protein concentrations (w/v) (0, 1, 2, 3, 4, 5, 7.5, 10, 12.5, 15, 20 and 25 mg/100 µl), for a total of BSA 96 solution samples (200 µl each). The eight McIlvaine buffer solutions (V = 7.5 ml each) were prepared as in from Na_2HPO_4 and citric acid as in [17].

Terahertz spectroscopy measurements.

For the THz spectroscopy measurements of samples we used a TPS Spectra 3000 (TeraView Limited, Cambridge) with an ATR module as previously described [16]. Briefly, 60 seconds measurements (1,800 scans acquired for each

measurement at a rate of 30 scans/s) with a 1.2 cm^{-1} resolution were performed under constant N_2 purge. TPS spectra 3000 software was used to process spectra by applying a three term Blackman–Harris apodisation function symmetrical about peak [16].

Fourier Transform Infrared (FTIR) spectroscopy measurements.

Using the Bruker Tensor 27 FT-IR with ATR accessory we measured the spectra in Mid-IR domain (400-4000 cm^{-1}) for the 96 solutions. The spectra had a sample scan time of 60 seconds and a 4 cm^{-1} resolution. The spectral data was processed using Opus software. The figures and the data statistical analysis were performed using Origin Pro and Excel software.

RESULTS AND DISSCUTIONS

FTIR spectroscopy

As a result of the experiments using FTIR spectroscopy, we obtained 96 spectra. When analyzing them we observed that the order of the buffers at the 8 pH values significantly changes according to the concentrations. At lower concentrations, the buffers at the two protein specific wavelenghts (~1654 cm^{-1} şi ~1539 cm^{-1}), are the smallest at pH 2.2, 3 and 6.

The buffers of the 8 pH values at 5% concentration are shown in Figure 1, At a concentration of 25 % (as in figure 2) the smallest buffers are met at pH 4, 5 and 7. At protein specific wavelenghts (~1654 cm^{-1} şi ~1539 cm^{-1}) the pure buffer aborbs the least at pH values of 7.4 and 2.2..

The figures 3, 4 and 5 show us the way FTIR spectrum of BSA solutions changes as the solutions concentration increases. Thus, at concentrations up to 10% amide II band is almost unobtrusive, but it reaches the highest values of the absorption in solutions with concentrations above 10%.

The highest values of the BSA solutions of 25% concentration are reached at pH values of 6, 7.4 and 8.

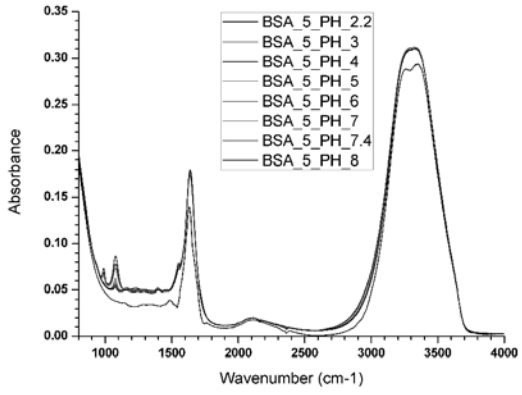

Fig 1. BSA FTIR 5%

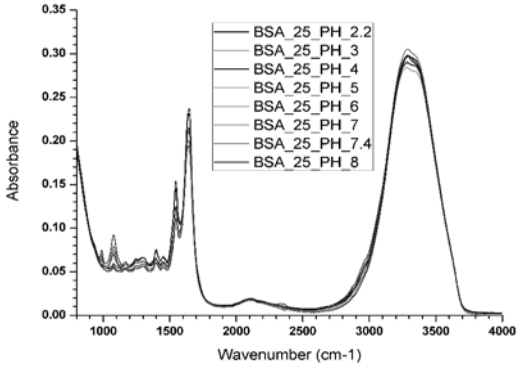

Fig 2. BSA FTIR 25%

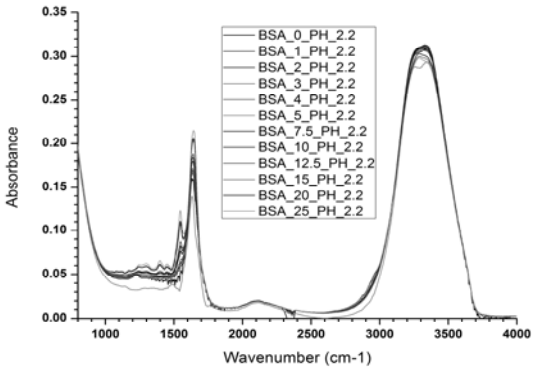

Fig. 3. BSA FTIR PH 2.2

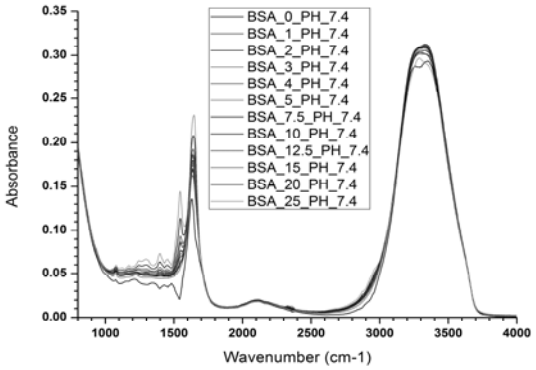

Fig. 4. BSA FTIR PH 7.4

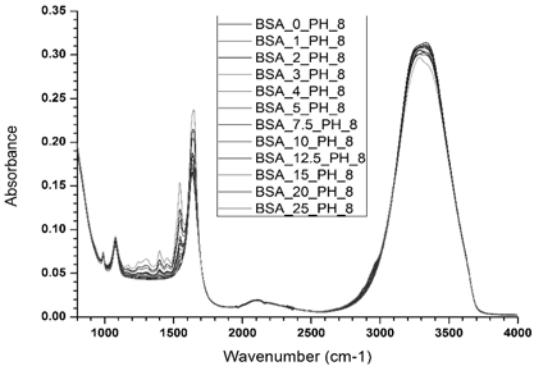

Fig. 5. BSA FTIR PH 8

THz spectroscopy

BSA solutions at the same pH values absorb differently depending on the concentration of protein in the sample.

The higher the protein concentration, the lower the THz radiation absorption due to the water content in the sample. Samples at a concentration of 10% (Fig. 6) show a higher THz absorption compared to solutions at a concentration of 25%. (Fig. 7). At pH 8 (Fig. 8) the solutions show a higher absorption of THz radiation than at pH 4 (Fig. 9). At all pHs, the buffer has a higher absorption level than the BSA samples in different degrees of concentration.

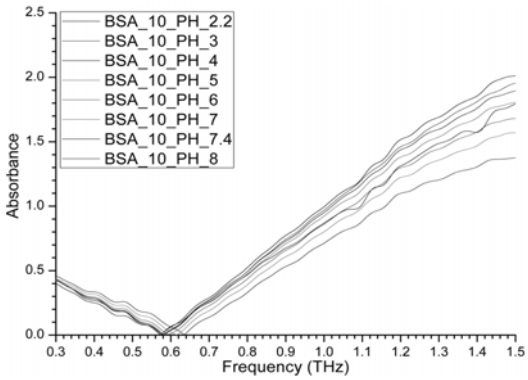

Fig. 6. BSA THz Concentration 10%

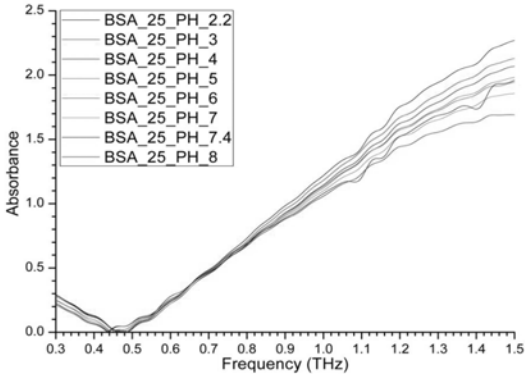

Fig. 7. BSA THz Concentration 25%

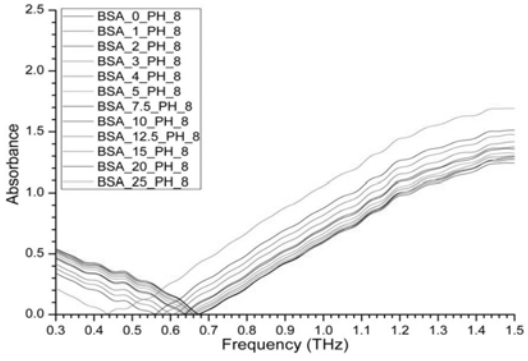

Fig. 8. BSA THz PH 8

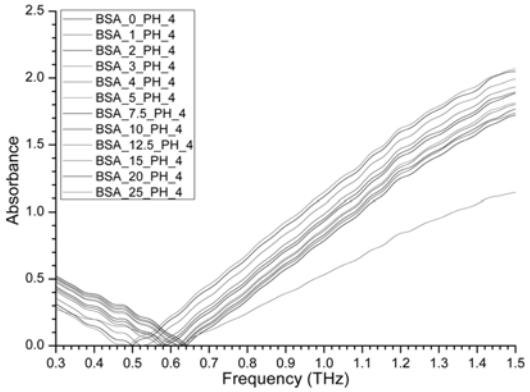

Fig. 9. BSA THz PH 4

In this experiment The pH 5 spectra showed the largest variation of absorbtion (Fig. 10).

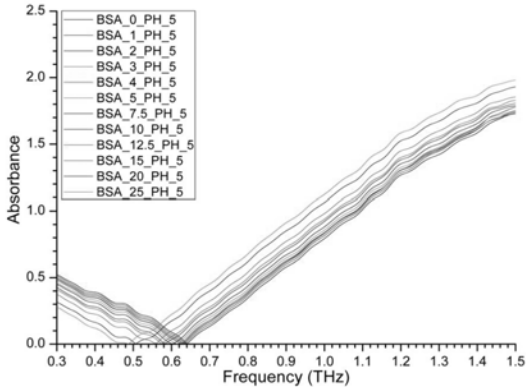

Fig. 10. BSA THz PH 5

CONCLUSIONS

The aim of this study was to investigate the changes induced by a wide range of pH and concentration values on the THz and FTIR spectra of bovine serum albumin (BSA) solutions.

After analyzing FTIR spectra we observed that the absorption at ~ 1654 cm $^{-1}$ and ~ 1539 cm^{-1}, nonlineary increases the BSA concentration at different pH values, fact which may be attributed to the crowded environment effect.

By using THz spectroscopy we were able to observe differences in absorption between BSA samples.

In this study we observed how the absorption spectrum of THz radiation changes as the concentration of the substance in the sample increases and also the pH variation of the solutions at different concentrations and pHs have specific absorption spectra..

Future experimental work will focus to investigate protein structure and flexibility using spectroscopy and molecular modeling techniques.

ABSTRACT

A natural biological environment involves highly concentrated molecular solutions. In such an environment, the activity of a protein depends on its structure and its conformational changes that appear when the protein interacts with other molecules. The purpose of this study is to elucidate the difference between the dilute and the high concentration solutions of bovine serum albumin (BSA) through THz and FTIR spectroscopy. We then measured the Mid-IR and THz spectra (400-4000 cm^{-1}) for the 96 solutions. Our data show that the absorption value increases in a nonlinear way with the BSA concentration at different pH values, a fact that may be assigned to the high concentration effect.

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