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# Specificity of amide VI band (FTIR) in globular protein stability

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**Abstract : Generally stabilization of globular proteins are attained by adding cosolvent glucose to resist denaturation due to extremes of pH in both acidic and alkaline region. In this work, the Fourier Transform Infrared (FTIR) spectra were recorded for ovalbumin protein with and without cosolvent (glucose) at pH 2, 5, 7, 9 and 12 in phosphate buffer. The changes in the secondary structures (α-helix, β-sheets and others) of ovalbumin caused by pH and influence of glucose on them were studied by FTIR spectra. These spectral examinations are specifically made in the amide VI band region towards the secondary structure of proteins since it was not analyzed yet. This analysis verify the stability of protein have good agreement with studies reported previously.**

**Keywords : Globular protein, denaturation, secondary structure, amide VI band.**

## Introduction

Globular proteins are transformed from their native states into different non-native state under pH variations. Maintenance or regaining the native state of globular protein is the prime factor in making use of a globular protein in food, pharmaceutical and other related industries[1]. The stability of protein can be achieved either by physical methods such as controlling the temperature or by chemical methods by adjusting the pH and/or the additives involved or by both. In present study stabilization of globular protein ovalbumin in the presence of glucose (cosolvent) is analyzed by Fourier Transform Infrared Spectroscopy (FTIR) at room temperature (303 K). This problem has been reported using ultrasonic and FTIR-amide I band in our earlier studies[2,3]. This spectral examinations secondary structure of globular proteins are made in the amide VI band region towards the secondary structure of globular proteins yet. This analysis verify the stability of globular protein have good agreement with studies reported previously.

FTIR is well appropriate experimental technique for the analysis of secondary structure of polypeptides and proteins[4]. Molecular vibrations are the fingerprint of structural organization of peptide chains which are tuned in to the hydrogen bonding environment and vibrations of the polypeptide backbone. Numerous vibration modes (amide I band, II, III and V bands) were previously reported to be perceptive to the secondary structure modifications of proteins and peptides[5–7]. But, the secondary structural modifications of globular proteins are not analyzed yet. In this aspect, we examine the globular protein stability due to variations in pH with and without addition of cosolvent. One of the food and pharmaceutically important globular protein ovalbumin is chosen as sample protein and glucose has been used as cosolvent in the current study. For the continuance of the required pH with steady ionic strength, phosphate buffers were prepared as suggested by Arda Alden Green[8] and used for extreme pH values as done by Waris *et al.*[9].

## Results and discussion

The primary FTIR spectra of ovalbumin systems were recorded for different pHs 2, 5, 7, 9 and 12 at 303 K are shown in Fig. 1 and Fig. 2. The best information from infrared protein spectra is obtained in the amide VI band, which appears in the region of 590 to 490 $cm^{-1}$. These

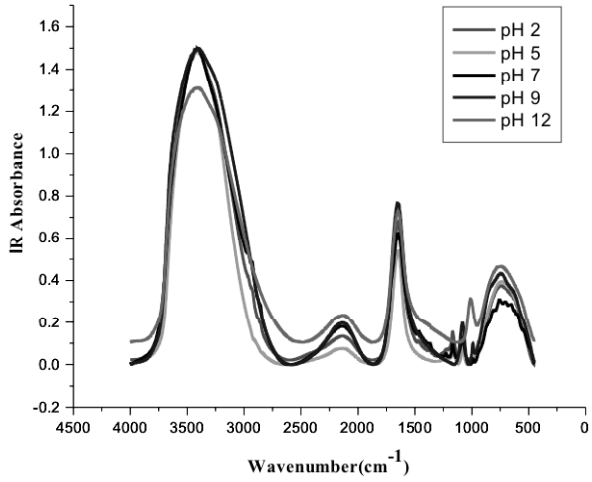


**Fig. 1.** FTIR primary spectra of phosphate buffer + ovalbumin system.

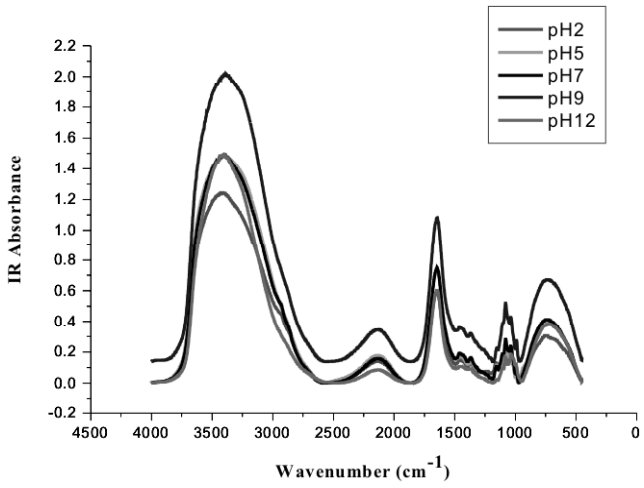


**Fig. 2.** FTIR primary spectra of phosphate buffer + ovalbumin system with cosolvent glucose.

bands arises mainly from bending motion of the C=O group of the peptide chain. The infrared second derivative spectra[11] permits direct quantitative analysis of the secondary structural components of proteins. Amide VI frequencies assigned to protein secondary structure are shown in Table 1[10]. Based on these peak assignments, the second derivatives and curve fitting of all spectra were performed by using Origin 7.0 program. Figs. 3 and 4 shows the second derivative curve fitted spectrum of ovalbumin system in amide VI band region for pH 2 and 7. Similar, the second derivatives were obtained for remaining pHs and hence to analyze the relative variations among the secondary structure components. The calculation of relative areas of the peaks leads to quantitative measurement for various secondary structure are consolidated for ovalbumin system in Table 2.

**Table 1.** Amide VI band frequencies[10]

| Sr. no. | Secondary structure | Frequency range ($cm^{-1}$) |
|---|---|---|
| 1. | α-Helix | 517–530 |
| 2. | β-Sheet | 540–545 |
| 3. | β-Turns | ~560–565 |
| 4. | Random coils | ~535 |

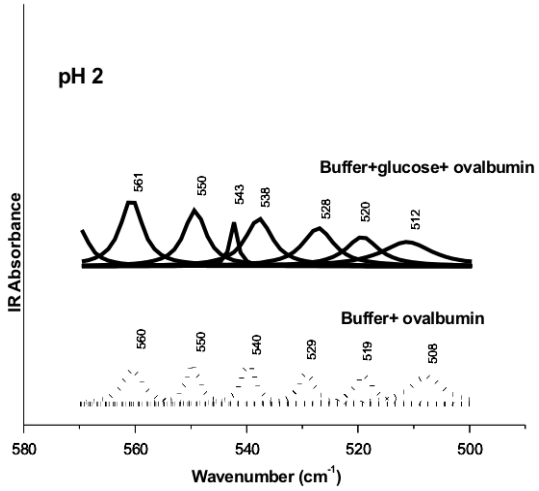


**Fig. 3.** Second derivative curve-fitted spectra at pH 2.

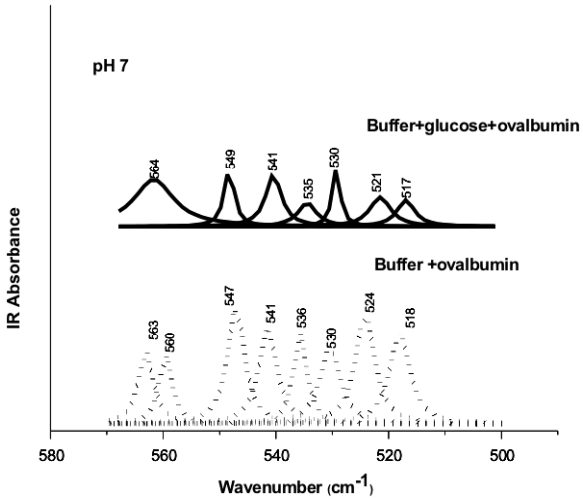


**Fig. 4.** Curve-fitted spectra at pH 7.

**Table 2.** Consolidated quantitative measurement of secondary structure

| pH | α-Helix (%) | | β-Sheet (%) | | β-Turns (%) | | Random coils (%) | |
|---|---|---|---|---|---|---|---|---|
| | I | II | I | II | I | II | I | II |
| 2 | 27 | 43 | 14 | 20 | 19 | 19 | 15 | 13 |
| 5 | 36 | 31 | 14 | 24 | 0 | 15 | 17 | 15 |
| 7 | 45 | 30 | 30 | 27 | 16 | 36 | 10 | 8 |
| 9 | 32 | 64 | 15 | 22 | 5 | 15 | 22 | 13 |
| 12 | 33 | 27 | 29 | 25 | 9 | 24 | 30 | 22 |

I – No cosolvent; II – with cosolvent.

The spatial arrangement of amino acid residues having a certain fraction of structural components referred as secondary structures. They are predominantly composed of α-helix, β-sheets, β-turns and random coils[12]. To validate the consistency of the observed results, as an example, the quantitative analysis of secondary structure of ovalbumin at pH 7 (Table 2) may be taken. It is observed that, in the present study, ovalbumin exhibits, in total, 45% α-helix, 30% β-sheet. These results are comparable with the values determined by FTIR in phosphate buffer-amide I region (32% α-helix, 41% β-sheet)[3]; (X-ray crystallography (~35% α-helix and ~45% β-sheet)[13,14]; FTIR in $D_2O$ by factor analysis (~36% α-helix and ~38% β-sheet)[15] and CD spectroscopy (~35% α-helix and ~44% β-sheet)[16]. It is seemed to be considerable deviation occurred in compared to previous reported results. This shows that amide VI region has less precise than amide I band spectra or in other direction the peak assignments need to be improved. However the present study is focused at analyze the structural stability of globular protein with addition of cosolvent at different pH environments rather than finding the percentage of secondary structural components of protein.

From Table 2, it is noticed that the decreasing trend of α-helix and β-sheet content at both acidic and alkaline pH extremes compared to pH 7. The reason for the above trend may be explained as follows : It is well known that β-sheet is stabilized by the hydrogen bond between NH and CO groups in different polypeptide strands, whereas in the α-helix, the hydrogen bonds are between NH and CO groups in the same strand. Globular proteins are transformed from their native states into different non-native state under various denaturing conditions such as extreme pH and elevated temperature. Further, it has been long known that at low pH 2, ovalbumin forms a partially folded molten-globule (MG) state in which the secondary structure is largely unaffected but most of the persistant tertiary structure is lost[17]. These above analysis suggest that ovalbumin got denatured at pH extremities. This result has good correlation with ultrasonic studies reported for ovalbumin[2].

It can be observed (Table 2) that α-helix and β-sheet contents are found to increase with addition of glucose especially in acidic pH extremity. This indicates that the addition of glucose makes to increase the intramolecular hydrogen bonding among the components of the system. However less effect has been observed at alkaline pH extremities.

As concern of β-turns, the increasing trend is perceived irrespective to pH. Fu *et al.*[18] reported that stability of protein can be increased by improving β-turn contents. This effect would provide good agreement with the observed result of β-turns especially at pH extremities, suggest that the addition of glucose tends to increase the formation turns content among the residues of the protein, thereby improves the stability against pH denaturation. As regards random coils, the decreasing trend is observed in approximately all pHs due to the addition of glucose. This indicates the stabilization of protein by adding cosolvent to the system.

The perusal of Table 2 shows that high content of random coils at pH extremities whereas feeble coils at pH 7. These results suggest that ovalbumin highly resembles with native state of protein at pH 7. They got changed into non-native states at high acidic and alkaline pHs and provide good correlation with the ultrasonic studies[2].

## Experimental

Ovalbumin protein purchased from Sigma Aldrich is used for sample preparation. 0.2 *M* aqueous solutions of both monobasic and dibasic sodium phosphates were mixed in different proportions to prepare phosphate buffers of pH 2, 5, 7, 9 and 12. Phosphate buffers were used as solvent for ovalbumin (5 mg/ml) is taken as system-I. 1 *M* solutions of glucose prepared in phosphate buffers of pH 2, 5, 7, 9 and 12 were used as solvent for ovalbumin (5 mg/ml) is taken as system-II.

The pH of these solutions was measured by the digital pH meter. After preparation, the stock solution was kept stored at 293 K overnight. These solutions were then degassed and each measurement was made after 20 min of thermal equilibration (303 $\pm$ 0.01 K). The FTIR spectra of proteins were recorded in the region 4000–450 $cm^{-1}$ by a Perkin-Elmer FTIR spectrometer at Sophisticated Analytical Instrument Facility Lab, Indian Institute of Technology, Madras, India.

## Conclusion

Amide VI band of FTIR spectra of ovalbumin provide admirable information about the conformational changes of protein under the variation of pH and also the effect of glucose on its stability. This task is accomplished by the estimation of secondary structure through curve-fitting of second derivative spectrum from its original spectra. This findings have good correlation with ultrasonic studies of ovalbumin protein.

### References


1. W. Chanasattru, E. A. Decker and D. J. McClements, *Food Hydrocolloids*, 2008, **22**, 1475.
2. L. Palaniappan and V. Velusamy, *Food Hydrocolloids*, 2013, **30**, 217.
3. V. Velusamy, PhD Thesis, Department of Physics, Manonmaniam Sundaranar University, 2013.
4. S. Y. Venyaminov and N. N. Kalnin, *Biopolymers*, 1990, **30**, 1259.
5. M. Fändrich and C. M. Dobson, *EMBO J.*, 2002, **21**, 5682.
6. M. Simonetti and C. Di Bello, *Biopolymers*, 2001, **62**, 109.
7. J. Kong and S. Yu, *Acta Biochim. Biophys. Sin.*, 2007, **39**, 549.
8. A. A. Green, *J. Am. Chem. Soc.*, 1933, **55**, 2331.
9. B. N. Waris, U. Hasan and N. Srivastava, *Thermochim. Acta*, 2011, **375**, 1.
10. Y. El-Khoury, R. Hielscher, M. Voicescu, J. Gross and P. Hellwig, *Vibrational Spectroscopy*, 2011, **55**, 258.
11. G. Damian and V. Canpean, *Romanian J. Biophys.*, 2005, **15**, 67.
12. S. Y. Lin, T. F. Hsieh and Y. S. Wel, *Peptides*, 2005, **64**, 543.
13. H. Loebermann, R. Tokuoka, J. Deisenhofer and R. J. Huber, *Mol. Biol.*, 1984, **177**, 531.
14. H. T. Wright, H. X. Qian and R. Huber, *J. Mol. Biol.*, 1990, **213**, 513.
15. S. J. Perkins, K. F. Smith, A. S. Nealis, P. I. Haris, D. Chapman, C. J. Bauer and R. A. Harrison, *J. Mol. Biol.*, 1992, **228**, 1235.
16. M. Bruch, V. Weiss and J. Engel, *J. Biol. Chem.*, 1988, **263**, 16626.
17. T. Koseki, N. Kitabatake and E. Doi, *J. Biochem.*, 1990, **107**, 389.
18. H. Fu, G. R. Grimsley, A. Razvi, M. Scholtz and N. Pace, *Proteins*, 2009, **77**, 491.