

## FTIR STUDY ON THE INTERACTION OF QUERCETIN AND AMANTADINE WITH EGG ALBUMIN

S. Bakkialakshmi\* and V. Barani

Department of Physics, Annamalai University, Annamalai nagar, Tamilnadu, India-608 002.

### ABSTRACT

The interaction of Quercetin & Amantadine with egg albumin was investigated using Fourier Transform Infrared (FTIR) technique. From this study it was proved that the drugs Quercetin [Q] and Amantadine [A] were bind well with Egg albumin [EA] and formed a new complex. This can be confirmed by Scanning Electron Microscope (SEM) Photographs.

**Keywords:** Egg albumin, Quercetin, Amantadine, FTIR, SEM

### INTRODUCTION

A variety of analytical tools is available to study protein conformation. Of these methods, Fourier Transform Infrared Spectrometry (FTIR) has proven to be the most versatile<sup>1-5</sup>. It allows analysis of protein conformation in a diverse range of environments, e.g. upon binding to membranes<sup>6-12</sup>, at the air-water interface<sup>13</sup>, in organic solvents<sup>14,15</sup>, or in the dehydrated state<sup>16-20</sup>.

The versatility of FTIR is based on the long wavelength of the radiation, which minimizes scattering problems. In addition, a wide range of sampling methods has been developed using transmission, (internal) reflection, or emission techniques. The choice of sampling method is mostly based on sample constraints. Although FTIR has become a common technique to investigate protein conformation, it is not without pitfalls<sup>1, 3, 21</sup>. It is common to compare infrared spectra of proteins recorded in different physical or with differing sampling techniques without investigating any potential effect of physical state (or) sampling technique on shape, position, and intensity of infrared absorption bands.

### MATERIALS AND METHOD

Egg Albumin, Amantadine and Quercetin were purchased from Sigma Aldrich company, Bangalore. The FTIR Spectra were recorded using

THERMO – SCIENTIFIC – i – D1 Transmission Spectrometer.

Scanning Electron Microscope (SEM) photographs of Egg Albumin without and with antiviral drugs were recorded using JOEL SEM MODEL, JSM – 5610 LV SCANNING ELECTRON MICROSCOPE.

### RESULTS AND DISCUSSION

Infrared spectroscopy has long been a simple and powerful tool for investigating the secondary structure of proteins. Fourier Transfer Infrared and Raman Spectroscopy are useful tools for studying the secondary structure of proteins quantitatively. The application of FTIR analysis for proteins has led to the identification of the folded protein and many findings on the folding mechanism<sup>22</sup>. Hydrogen bonding and the coupling between transition dipoles are the most important factors governing conformational sensitivity of the amide bands. Both the protein amide I band  $\sim 1653\text{ cm}^{-1}$  (mainly C = O) stretch) and the amide II band  $\sim 1548\text{ cm}^{-1}$  (C – N Stretch coupled with N – H bending mode) have a relationship with the secondary structure of protein, but the amide I band is more sensitive to change in the secondary structure of protein than amide II band. In the IR region, due to amide I and amide II vibrations the frequencies of the band are sensitive to the secondary structure of proteins. The amide I vibration mode originates from the C = O

stretching vibration of the amide group. The recorded FTIR spectra of EA, (EA + A) and (EA + Q) are shown in figs 1, 2 & 3 respectively. The change in FTIR absorption peak intensities only have been observed. It can be concluded that only 1% to 28% weaker complexes were formed. These are tabulated in table 1 & 2 respectively. Scanning electron microscope (SEM) photographs of Egg albumin, Egg albumin with Amantadine and

Quercetin are shown in figs 4, 5 & 6 respectively. The powdered form of Egg albumin and the mixture of Egg albumin with Amantadine and Quercetin were separately scanned through SEM. The different morphologies that could be seen in figures support the binding of antiviral drugs with EA. This is proof enough of the binding of antiviral drugs with Egg Albumin.

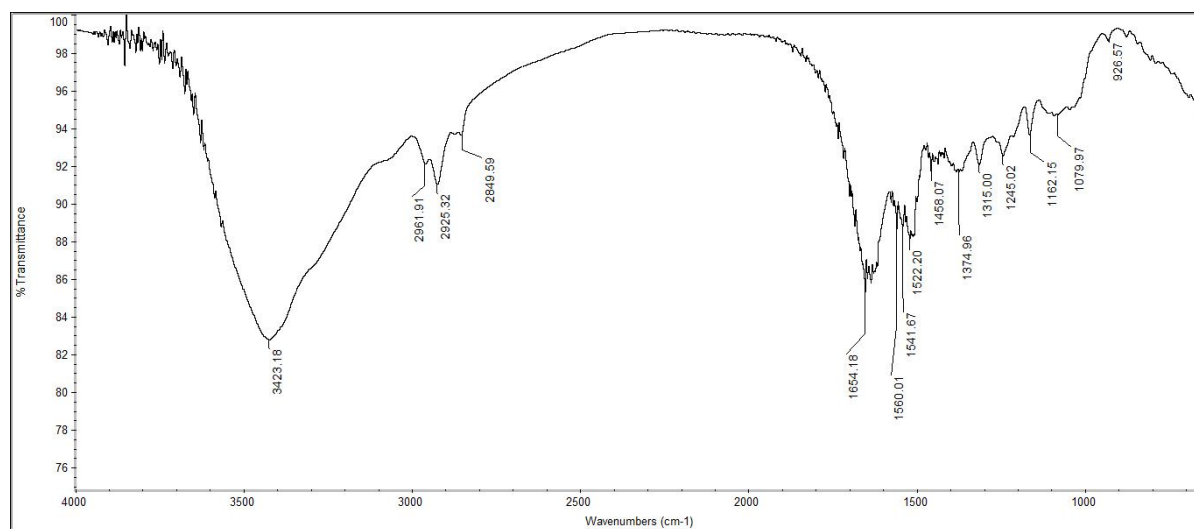


Fig. 1: FTIR Spectra of Egg Albumin

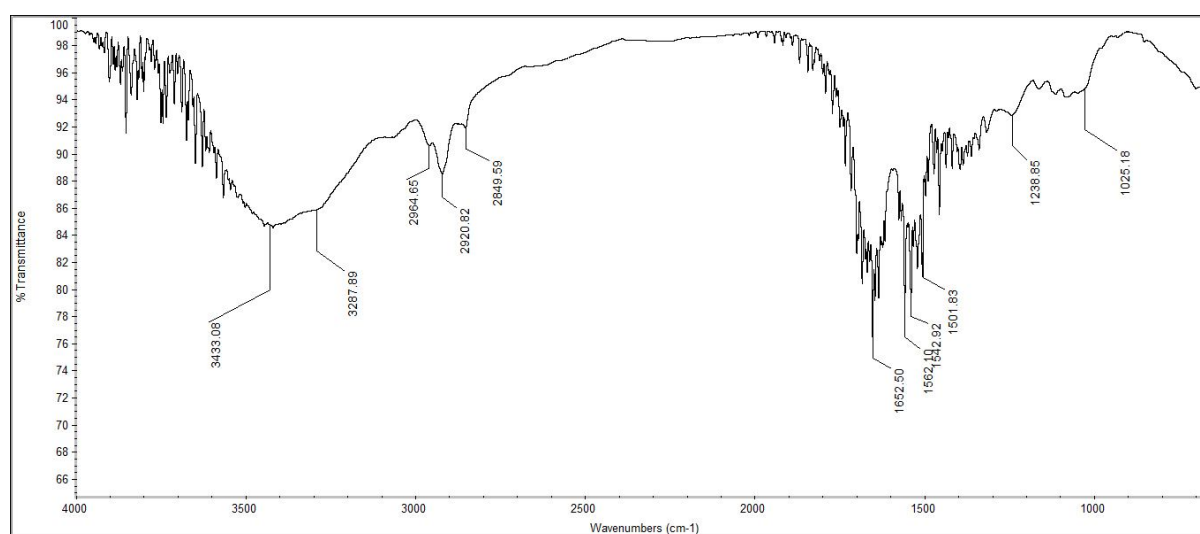


Fig. 2: FTIR Spectra of Egg Albumin with Amantadine

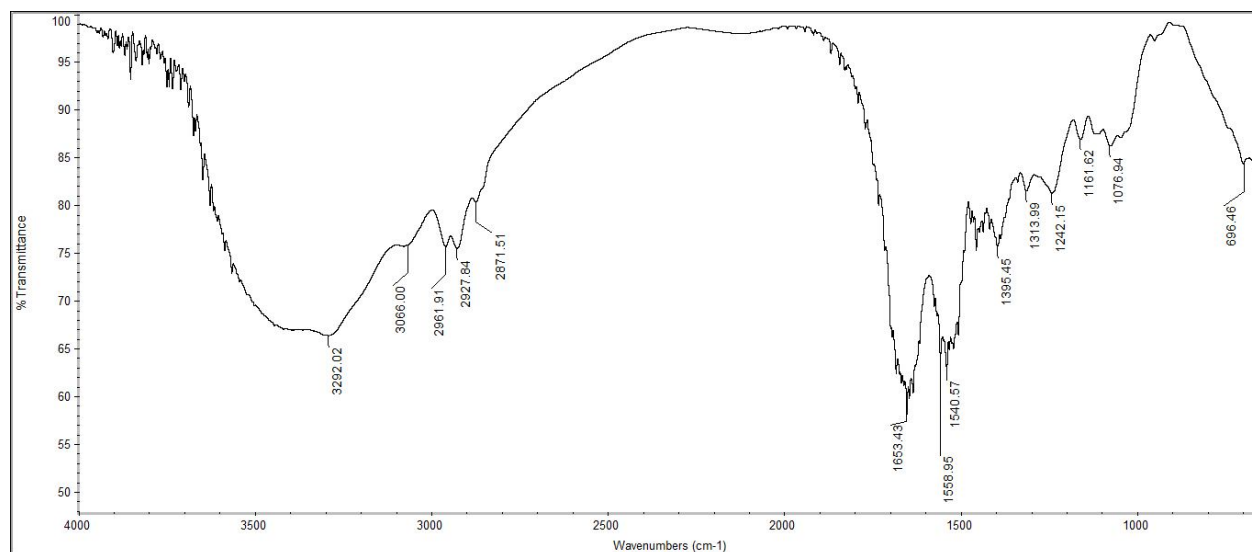


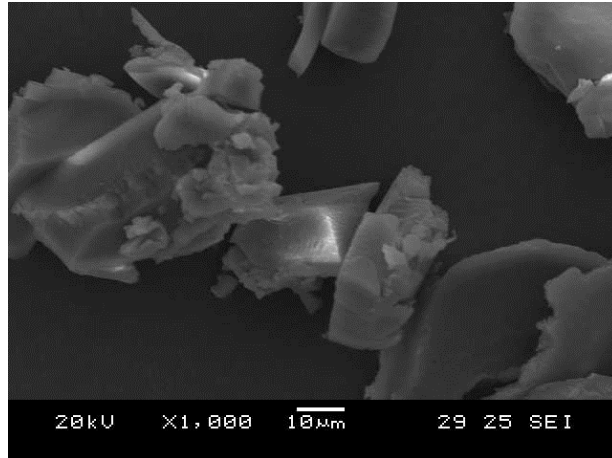
Fig. 3: FTIR Spectra of Egg Albumin with Quercetin

Table 1: Differences in FTIR absorption peak intensities of EA and Amantadine before and after complex formation

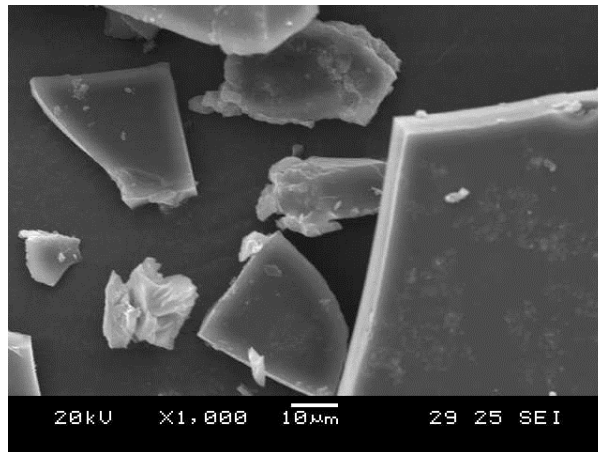
Intensities (cm <sup>-1</sup> )		Difference in intensities prior t and after (%)	Tentative assignment
EA	EA + A		
3423.18	3433.08	1	O – H stretching
2961.91	2964.65	2	C – H stretching
2925.32	2920.82	3	C – H stretching
2849.59	2849.59	2	C – H stretching
1654.18	1652.50	8	C = N stretching
1560.01	1562.10	6	C = O stretching
1541.67	1542.92	7	C = O stretching

Table 2: Differences in FTIR absorption peak intensities of EA and Quercetin before and after complex formation

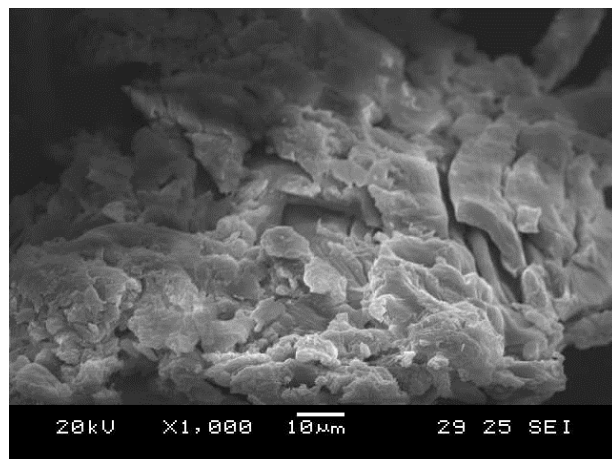
Intensities (cm <sup>-1</sup> )		Difference in intensities prior t and after (%)	Tentative assignment
EA	EA + Q		
2961.91	2961.91	17	C – H stretching
2925.32	2927.84	16	O – H stretching
2849.59	2871.51	14	C – H stretching
1654.18	1653.43	28	C = N stretching
1560.01	1558.95	22	C = O stretching
1541.67	1540.57	25	C = O stretching
1315.00	1313.99	10	C – O stretching
1245.02	1242.15	10	C – O – H stretching
1162.15	1161.62	7	C – O stretching
1079.97	1076.94	9	C – O – H stretching



**Fig. 4: Scanning Electron Microscope (SEM) Photograph of Egg albumin**



**Fig. 5: Scanning Electron Microscope (SEM) Photograph of Egg albumin with Amandadine**



**Fig. 6: Scanning Electron Microscope (SEM) Photograph of Egg albumin with Quercetin**

**CONCLUSION**

FTIR and SEM analyses were successfully carried out to prove the binding of Quercetin and Amantadine with Egg Albumin. Two new complexes were formed (1) Egg Albumin with Quercetin and (2) Egg albumin with Amantadine.

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