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Research Paper

INTERACTION STUDY OF EGG ALBUMIN WITH TWO ANTIVIRAL DRUGS BY FTIR

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The interaction of Quercetin with egg albumin in SPAN40 solution was investigated using Fourier Transform Infrared (FTIR) technique and Scanning Electron Microscope (SEM). 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.

Keywords: Egg albumin, Quercetin, FTIR, SEM

INTRODUCTION

Egg albumin is the major protein constituent of egg whites. Egg albumin is a phosphorylated-glycoprotein. From the amino acid sequence, the peptide portion of the molecule consists of 385 residues and has a molecular weight of 42.7 kDa. The SPAN 40 are a range of mild non-ionic surfactants providing formulating benefits in a number of home care applications. As non-ionics, SPAN 40 offer many advantages over ionic surfactants including increased stability, formulating flexibility and wides rcompatibility (Haris, 2000; Haris and Servercan, 1999; Jackson, 1995). They are stable in mild acids, alkalis and electrolytes and do not react with ionic ingredients or actives.

In general, surfactants – protein interactions are not well understood, and most comprehensive studies use ionic surfactants (e.g., SDS, CTAB) since, interactions are stronger and interpretation of results is somehow easier (Singh and Fuller, 1991). However, the surfactants that are normally used in formulations are non-ionic, for their stabilizing properties, while ionic surfactants can also bind to oppositely charged polar groups in proteins and cause denaturation. In particular, physico-chemical studies on surfactants used for pharmaceutical formulations are needed (Gericke *et al.*, 1997; Goormagtigh *et al.*, 2004). Since most reports are focused on their use as protein denaturing agent, on their effect on the competitive adsorption at fluid interfaced, and on their relevance in the stabilization of emulsions and foams in the food industry.

FTIR spectroscopic measurements were made to assess the effect of quercetin on the

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molecular component of the structure of EA. Infrared spectroscopy has long been the simple and powerful method for investigating the secondary structure of proteins. In the IR region, the frequencies of the band due to the amide I and II vibrations are sensitive to the secondary structure of proteins (Jackson, 1998; Marsh, 1999). Today, an individual would be hard-pressed to find any science field which does not employ methods and instruments based on the use of fine focussed electron and ion beams, well instrumented and supplemented with advanced methods and techniques. SEM provides possibilities not only of surface imaging but quantitative measurement of object topologies, local electro physical characteristics of semiconductor structures and performing elemental analysis (Schladitz, 1999; Carpenter, 1998).

MATERIALS AND METHODS

Egg Albumin, Quercetin and in SPAN40 were purchased from Sigma Aldrich Company, Bangalore. The FTIR Spectra were recorded using Thermonicolet iS_5 --FTIR spectrophotometer.

Scanning Electron Microscope (SEM) photographs were recorded using JEOL SEM MODEL, JSM-5610 LV SCANNING ELECTRON MICROSCOPE with an accelerating voltage of 20 KV, at high vacuum (HVF) mode and Secondary Electron Image (SEI).

RESULTS AND DISCUSSION

Infrared spectroscopy has long been a simple and powerful tool for investigating the secondary structure of proteins. Fourier Transform 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. Hydrogen bonding and the coupling between transition dipoles are the most important factors governing conformational sensitivity of the amide bands (Griebenow, 1995; Carpenter *et al.,* 1998).

Both the protein amide I band at 1653 cm^{-1} (mainly C = O) stretch) and the amide II band at 1548 cm^{-1} (C – N Stretch coupled with N –H

Figure 1: FTIR Spectra of EA

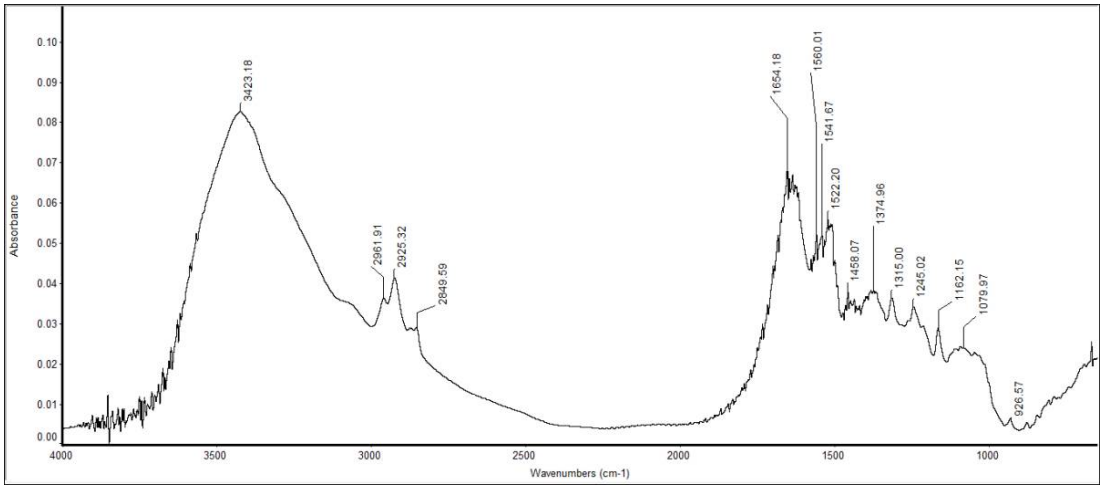


Figure 2: FTIR spectra of EA + Quercetin

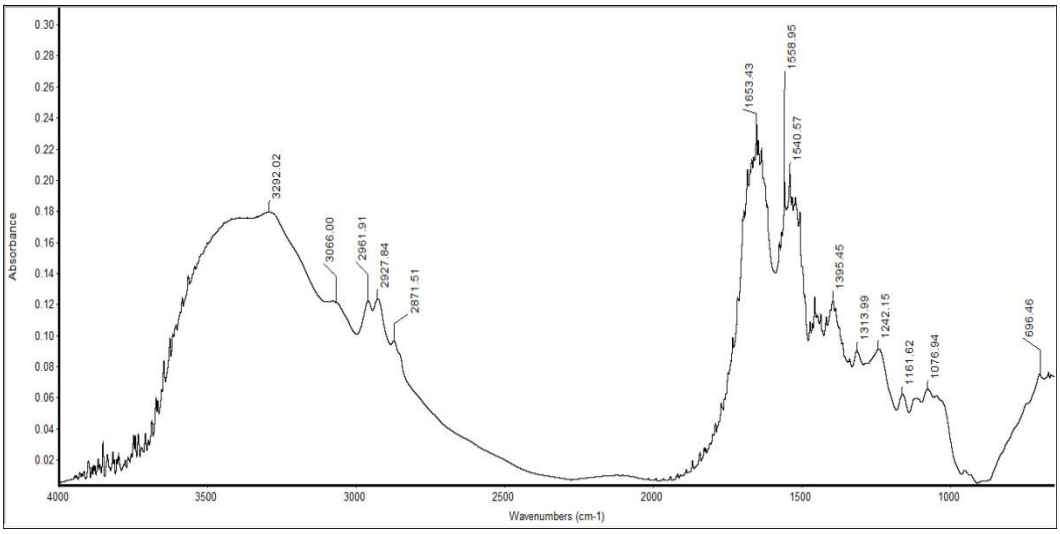
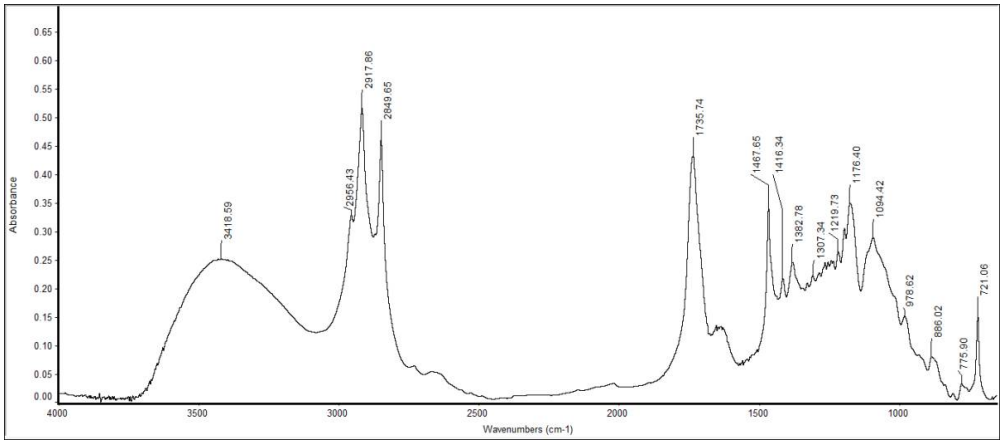


Figure 3: FTIR Spectra of EA with Quercetin in SPAN 40



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 + Q) in SPAN40 are shown in (Figures 1, 2 and 3) respectively (Prestrelski *et al.,* 1993). The change in FTIR absorption peak intensities only has been observed. It can be concluded that only 1% to 28% weaker complexes were formed. These are tabulated in (Table 1) respectively.

Egg albumin in SPAN 40 was powdered separately and the structure of their particles in

Table 1: Differences in FTIR Absorption Peak Intensities of EA and Quercetin in SPAN40 Before and After Complex Formation

Intensities (cm^{-1})			Difference in Intensities Prior t and After (%)	Tentative Assignment
EA	EA+Q	EA + Q + SPAN 40		
2961.91	2961.91	3418	17	C – H stretching
2925.32	2927.84	2956	16	O – H stretching
2849.59	2871.51	2917	14	C – H stretching
1654.18	1653.43	2849	28	C = N stretching
1560.01	1558.95	1736	22	C = O stretching
1541.67	1540.57	1468	25	C = O stretching
1315.00	1313.99	1416	10	C – O stretching
1245.02	1242.15	1383	10	C – O – H stretching
1162.15	1161.62	1219	7	C – O stretching
1079.97	1076.94	1176	9	C – O – H stretching

Figure 4: SEM Image of EA

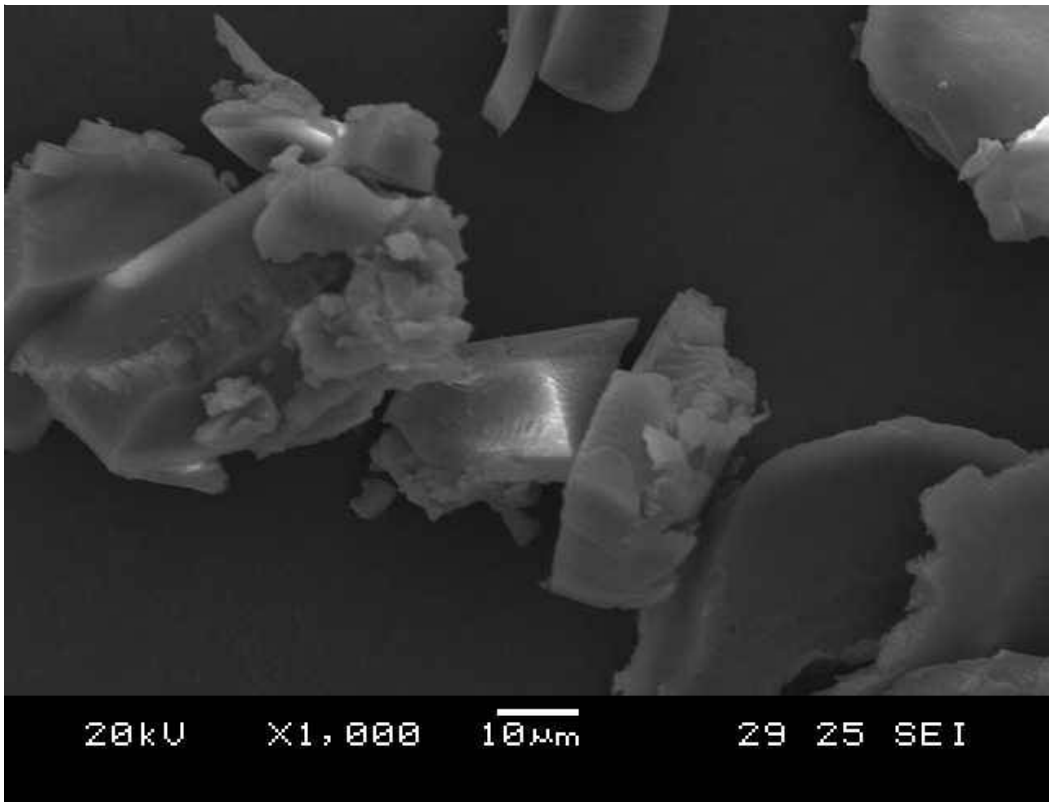


Figure 5: SEM image of EA + Quercetin

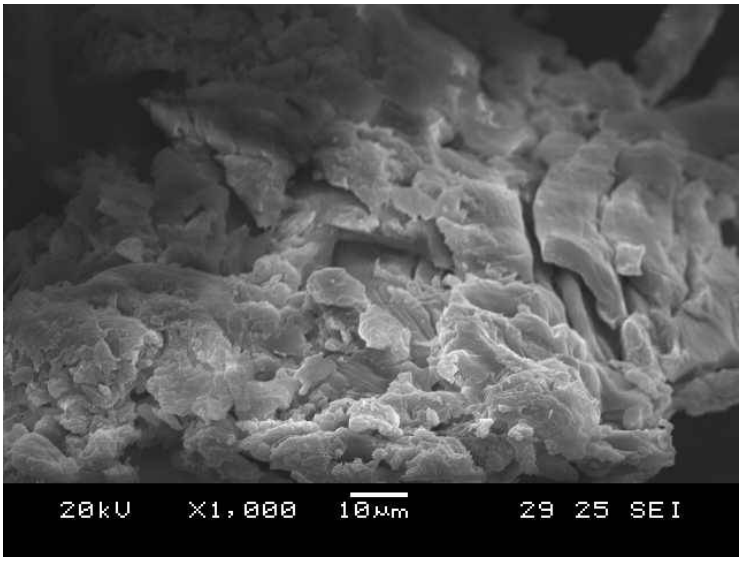
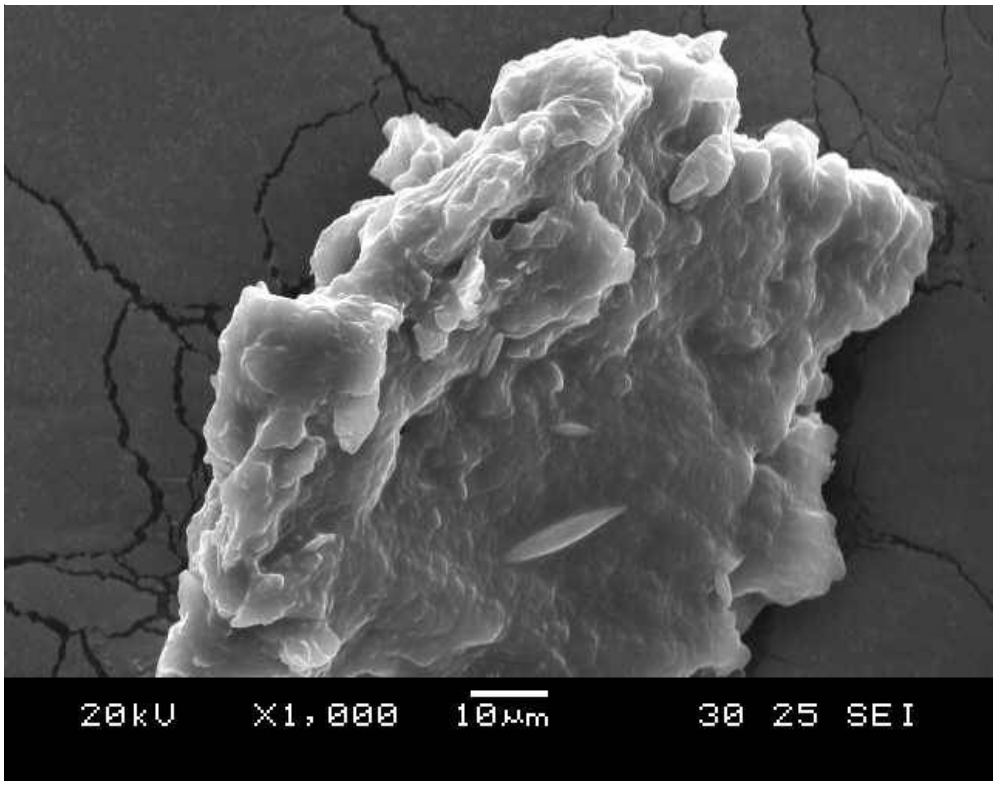


Figure 6: SEM Image of EA with Quercetin in SPAN 40



this powder was observed first, in the scanning electron microscope (Figures 4, 5 and 6).Then the particles of the powdered form of the complexes were also studied (Kendrick *et al.,* 1996). The structure of the particles of the complexes EA without quercetin appear different from that of Egg albumin with quercetin in SPAN 40 and it can be assumed as a proof of the formation of new complex.

CONCLUSION

FTIR was successfully carried out to prove the binding of Quercetin with Egg Albumin in SPAN40. Two new complexes were formed: (1) Egg Albumin with Quercetin; and (2) Egg albumin in SPAN40 solution.

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