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RESEARCH ARTICLE

LIFE-TIME MEASUREMENTS USING FLUORESCENCE SPECTROSCOPY

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ABSTRACT

Life time measurements for Egg Allumin [EA] with two different anti-viral drugs Amantadine [A] and Quercetin [Q] in SPAN40 have been carried out by Time resolved fluorescence spectrofluorimeter. The decay parameters have been calculated. The average lifetime (τ) and χ^2 values have also been calculated and tabulated.

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INTRODUCTION

Serum albumin is one of the most abundant proteins, and plays an important role in the transport and deposition of a variety of ligands in blood (Feshke *et al.,* 1981; Vallner, 1977; Carter and Ho, 1994; He, 1992). Albumin is a class of simple, water – soluble proteins that are found in egg white, blood serum, milk and many other animal and plant fluids and tissues. Albumin has been used as the subject of many investigations because of its important roles in maintaining normal biochemical functions. A number of biochemical and molecular biological investigations have been revealed that proteins are frequently the "targets" for therapeutically active flavonoids of both natural and synthetic origins (Lamson *et al.,* 2000). Flavonoids are common constituents of plants and therefore have been identified in a broad range of fruits and vegetables as well as in beverages such as tea, red wine, coffee, and beer. Flavonoids particularly quercetin derivatives have received more attention as dietary constituents during the last few years (Dehghan *et al.,* 2010). Many studies showed that flavonoids have a wide range of biological activities, such as anticancer, antiviral, antibacterial, antioxidants and anti-inflammatory effects (Wang *et al.,* 2008; Nafisi *et al.,* 2008; Nafisi *et al.,* 2009; Barriyanga and Tajmir-Riahi, 2009). Quercetin is known to be a complex with various metal cations to form stable compounds which have demonstrable antibacterial properties and anti-tumour activity (Zhou *et al.,* 2001; Bravo and Anacona, 2001; Zhou *et al.,* 2001; Kanakis *et al.,* 2006; Prud`home *et al.,* 1997; Sweet *et al.,* 1997; Choi *et al.,* 2009). The most abundant naturally occurring flavonoid, quercetin inhibits the activity of enzymes such as calcium phospholipid- dependent protein kinase (Kanakis *et al.,* 2006) among others. Amantadine is an antivirotic drug that has been used to treat influenza Parkinson disease (Prud`home *et al.,* 1997; Sweet *et al.,* 1997; Choi *et al.,* 2009; Kuno, 2009; Brenne *et al.,* 1989; Nishikawa *et al.,* 2009). Although amantadine is effective as a preventative prophylaxis and as a treatment of the influenzaA viral infections, its clinical use as and to anti-influenza drug is limited due to its central nervous system side effects (Skehel, 1992). Amantadine (1-amino Admantine) is used against infection with influenza type A virus and to ameliorate symptoms when administered during the early stages of infection (Prud`home *et al.,* 1997), as well as in the management of herpes zoster (Martindale, 2002). Amantadine has mild antiparkinsonism activity and also has been used in the management Parkinsonism mainly in early disease stage when symptoms are mild. Amantadine is usually given by mouth as the hydrochloride salt (Martindale, 2002).

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MATERIALS AND METHODS

Egg Albumin, Quercetin, Amantadine and SPAN40 were purchased from Sigma Aldrich Company Bangalore. Double – distilled water was used in all the experiments. Fluorescence life time measurements were carried out in a Hariba – Jobin Yvon [spex-sf 13-11] spectrofluorimeter.

RESULTS AND DISCUSSION

Fluorescence life time deals as a sensitive parameter for analyzing excited state interactions, the local environment around the fluorophore (Prendergast, 1991) and on the nature of binding between probe and protein (Wang *et al.,* 2005). Lifetime measurements of Egg Albumin in SPAN40 were also performed at different molar ratios of Amantadine and Quercetin. The decay parameters, average fluorescence lifetime (τ) and χ^2 values are listed in Table 1 and Table 2. The average life time (τ) was calculated by following relation (Eq1)

$$<\tau>=\frac{\alpha_1\tau_1^2+\alpha_2\tau_2^2+\alpha_3\tau_3^2}{\alpha_1\tau_1+\alpha_2\tau_2+\alpha_3\tau_3} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where τ_1, τ_2 and τ_3 are the decay times and a_1, a_2 and a_3 are the pre exponential factors.

Fig 1 and Fig 2. Show the fluorescence decay of Egg Albumin in the presence and absence of Amantadine and Quercetin. Average Lifetime values have been calculated and are presented in Tables 1& 2, along with Relative amplitude values and χ^2values have been calculated.

Table 1. Fluorescence lifetime and Relative amplitudes of EA with different concentrations of Amantadine in SPAN40

Concentration of Amantadine M	Lifetime (ns)			Average life time <τ> ns	Relative amplitude			χ^2	S.D x 10^{-11}Sec		
	τ_1	τ_2	τ_3		B_1	B_2	B_3		τ_1	τ_2	τ_3
0	2.82	6.27	3.48	5.06	28.88	63.56	7.56	1.31	9.28	2.74	3.49
0.2	2.25	6.40	5.41	4.71	30.39	62.34	7.27	1.23	8.16	2.72	3.35
0.4	2.12	6.42	5.68	4.70	28.58	63.42	8.00	1.25	9.51	2.59	3.57
0.6	2.26	6.52	5.73	4.64	31.28	59.47	9.25	1.37	8.61	2.99	2.86

Table 2. Fluorescence lifetime and Relative amplitudes of EA with different concentrations of Quercetin in SPAN40

Concentration of Quercetin M	Lifetime (ns)			Average life time <τ> ns	Relative amplitude			χ^2	S.D x 10^{-11}Sec		
	τ_1	τ_2	τ_3		B_1	B_2	B_3		τ_1	τ_2	τ_3
0	2.18	6.27	3.48	5.06	28.88	63.56	7.56	1.31	9.28	2.74	3.49
0.2	2.15	6.30	4.82	4.59	30.11	61.71	8.18	1.27	8.54	2.74	2.80
0.4	1.94	6.20	3.11	4.51	29.40	63.20	7.40	1.19	5.90	2.35	2.66
0.6	1.86	6.11	1.54	4.34	27.56	61.91	10.53	1.17	4.63	2.20	1.62

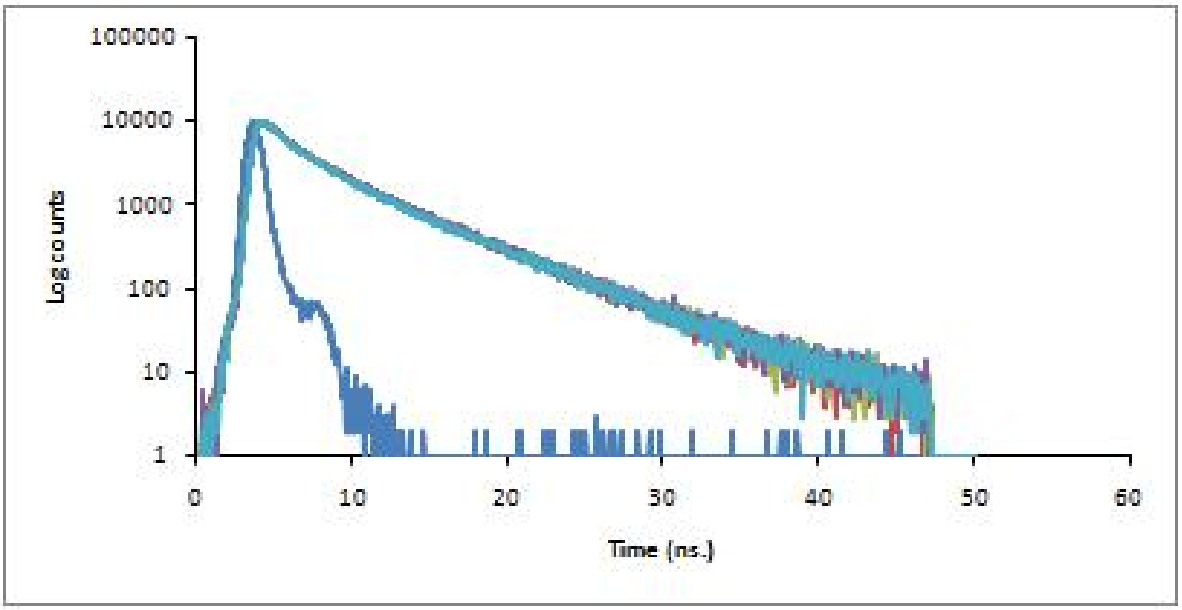

Fig.1. Time-resolved fluorescence spectra of EA with different concentrations of Amantadine in SPAN40 (mol L^{-1}) (1) 0, (2) 0.2, (3) 0.4, (4) 0.6

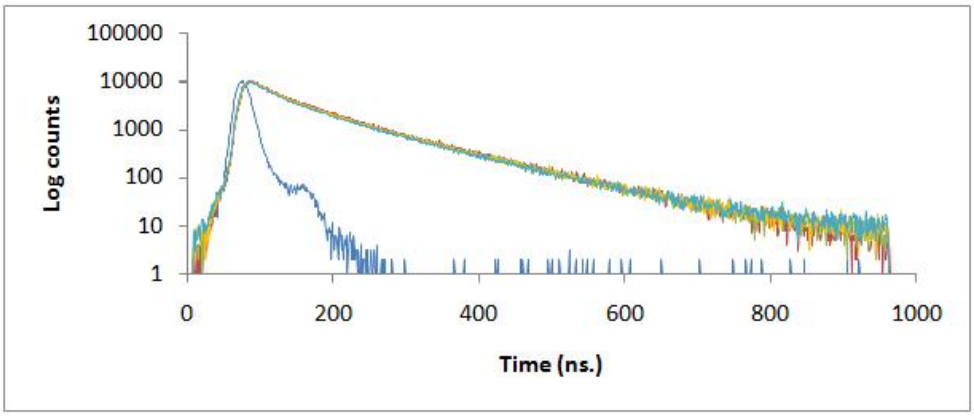

Fig.2. Time-resolved fluorescence spectra of EA with different concentrations of Quercetin in SPAN40 (mol L^{-1}) (1)0, (2)0.2, (3)0.4, (4) 0.6

Conclusion

Time-resolve fluorescence spectra have been recorded without and with Quercetin and Amantadine for egg albumin in SPAN40 solution successfully. The calculated average lifetime values have been tabulated.

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