KINETICS OF OXIDATION OF THIAMINE BY PHOSPHATE RADICAL ANIONS PROTECTION BY CAFFECIC ACID

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ABSTRACT:

The oxidation of thymidine by $PO_4^{\bullet-}$ has been followed by measuring the absorbance of thymiine at 264 nm to 310 nm spectrophotometrically. The rates and the quantum yields (φ) of oxidation of thymine by phosphate radical anion have been determined in the presence of different concentrations of caffeic acid. Increase in [caffeic acid] is found to decrease the rate of oxidation of thymine suggesting that caffeic acid acts as an efficient scavenger of $PO_4^{\bullet-}$ and protects thymine from it. Phosphate radical anion competes for thymine as well as for caffeic acid. The quantum yields of photooxidation of thymine have been calculated from the rates of oxidation of thymidine and the light intensity absorbed by Peroxodiphosphate (PDP) at 264 nm, the wavelength at which PDP is activated to sulphate radical anion. From the results of experimentally determined quantum yields (φexptl) and the quantum yields calculated (φcal) assuming caffeic acid acting only as a scavenger of $PO_4^{\bullet-}$ radicals show that φexptl values are lower than φcal values. The experimentally found quantum yield values at each caffeic acid concentration and corrected for $PO_4^{\bullet-}$ scavenging by caffeic acid (φ1) are also found to be greater than φexptl values. These observations suggest that the thymine radicals are repaired by caffeic acid in addition to scavenging of phosphate radical anions.

Keywords: Kinetics, thiamine hydrochloride (vit – B_1), PDP and caffeic acid.

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Introduction :

It is known that hydrocinnamic acids are natural anti oxidants and their anti oxidant and antifungal activity is mainly due to their ability to scavenge several oxidising free radicals In recent times focus is on the protective[1-4] action of naturally occurring ant oxidant on biologically important molecules and in this connection studies involving caffeic acid assume importance due to its wide spread occurrence in nature[5].

DNA damage due to ionizing radiation can be a direct (or) an indirect effect[6-8]. In order to mimic and understand the mechanism[9-10] of direct effect of ionizing radiation in DNA model compounds and to understand the mechanism of protection from phosphate radical anion (PO_4^{2-}) we have carried out a systematic kinetic study of Oxidation of thiamine by PO_4^{2-} in the presence of varying concentration of caffecic acid.

Materials and Methods:

Thiamine was from E merck Germany Caffecic acid was from sigma, USA. Lithium salt of peroxydiphosphate was prepared by electrolysis method. Irradiation were per formed at Room temperature (25^{o}C) with a 400 W, medium pressure mercury lamp using quantum yield reactor. All solutions were prepared using double distilled water and standardised by cerimetry using ferroin indicator PDP solution was added to measure excess of ferrous ammonium sulphate and back fit rated with standard cericammonium sulphate solution.

Results and discussion:

The oxidation of thiamine by phosphate radicals has been carried out by irradiating the reaction mixture containing known concentration of thiamine and PDP. The reaction was followed by measuring the absorbance at 264nm and 310nm with time.

Table 1: Effect of [thiamine] on Oxidation of thiamine in presence of PDP.

[Thiamine] – 5 X 10^{-5}M

[PDP] – 2 X 10^{-3}M

λ= 264 nm

Irradiation time (In minutes)	Absorbance	Log absorbance	1+ log absorbance
0	0.367	-0.435	0.265
1	0.359	-0.445	0.555

2	0.353	-0.452	0.548
3	0.346	-0.461	0.539
5	0.329	-0.483	0.517
7	0.319	-0.496	0.504
9	0.299	-0.524	0.476
11	0.283	-0.548	0.452
13	0.263	-0.580	0.42
16	0.238	-0.623	0.377
19	0.212	-0.674	0.326

Slope = -0.00815

K_{abs} = -0.00815×2.303 = -0.001876945

The initial rates of oxidation of thiamine were calculated from the plots of absorbance verses time using micro cal origin computer program on a personal computer. The quantum yields of oxidation of thiamine were calculated from the initial rates of oxidation of thiamine and light intensity at 264 nm. The rates and quantum yields of oxidation of thiamine increased with increase in concentration of thiamine.

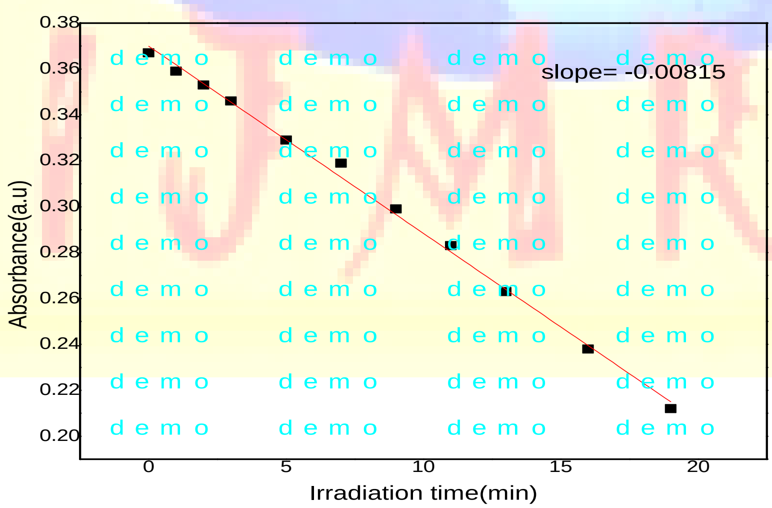


Fig.1: Effect of [Thiamine] on oxidation of Thiamine in presence of PDP

Table 2:

[Thiamine] – 2 X 10^{-5}M

[PDP] – 2 X 10^{-3}M

Irradiation time (In minutes)	Absorbance	Log absorbance	2+ log absorbance
0	0.144	-0.416	1.1584
1	0.142	-0.8477	1.1523
2	0.138	-0.86.1	1.1399
3	0.131	-0.8827	1.1173
5	0.115	-0.9393	1.0607
7	0.101	-0.9956	1.0044
9	0.088	-1.0555	0.945
11	0.07	-1.1249	0.8751

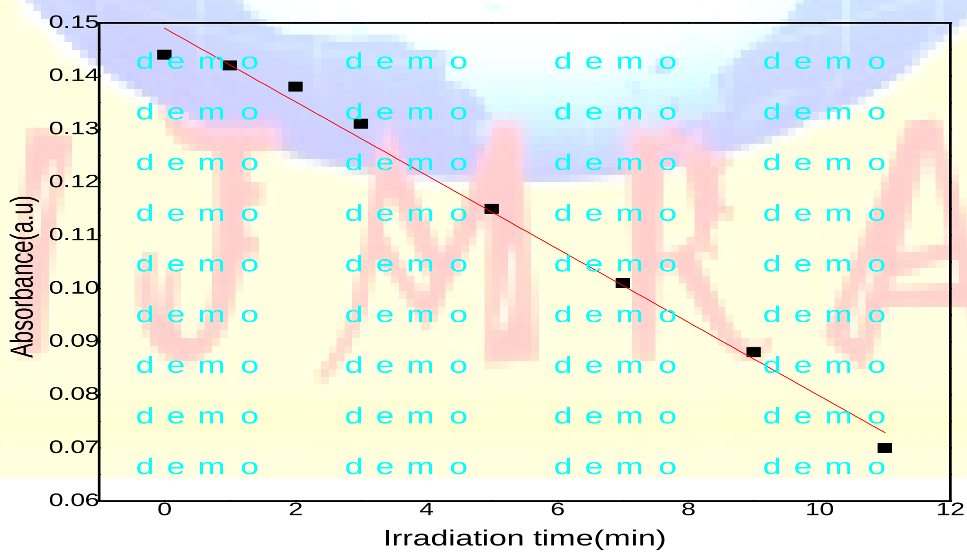


Table 3:

[Thiamine] – 8 x 10^{-5}M

[PDP] – 2 x 10^{-3}M

λ = 310nm

Irradiation time (In minutes)	Absorbance	Log absorbance	1+ log absorbance
0	0.566	-0.244	0.753
1	0.559	-0.253	0.747
2	0.551	-0.259	0.741
3	0.541	-0.267	0.733
5	0.528	-0.277	0.723
7	0.513	-0.290	0.710
9	0.494	-0.306	0.694
11	0.481	-0.318	0.682
13	0.456	-0.341	0.659
16	0.425	-0.372	0.625
19	0.394	-0.404	0.596

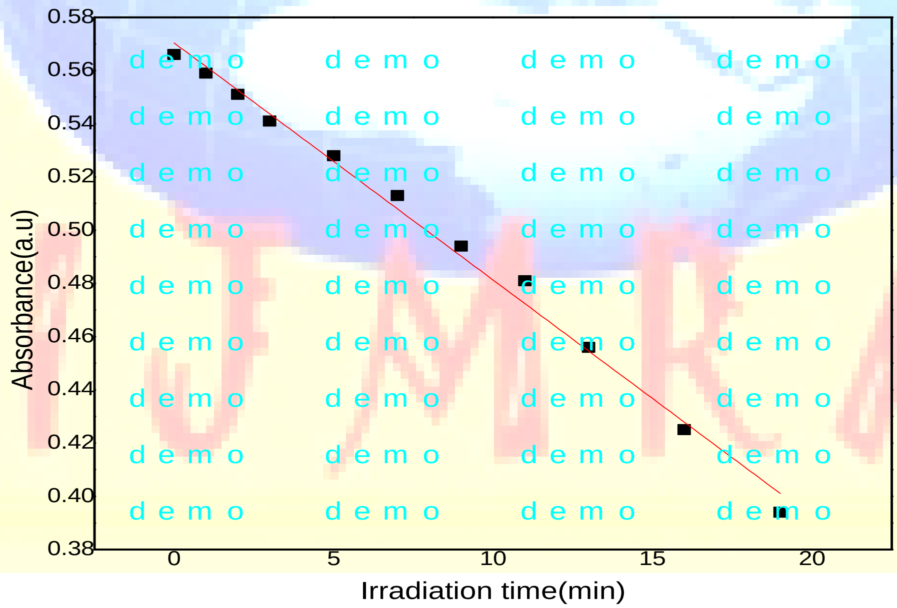


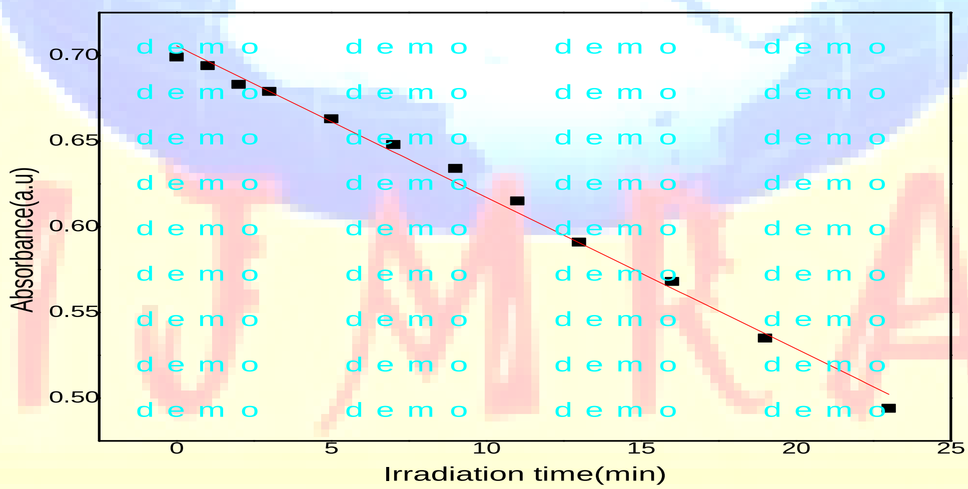
Table 4:

[Thiamine] – 1 x 10^{-4}M

[PDP] – 2 x 10^{-3}M

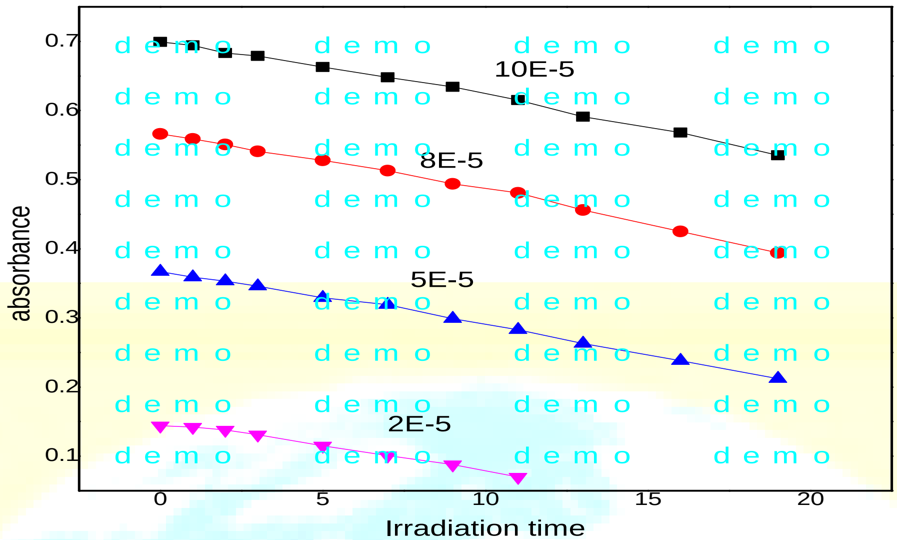
λ= 310nm

Irradiation time (In minutes)	Absorbance	Log absorbance	1+ log absorbance
0	0.699	-0.155	0.845
1	0.694	-0.165	0.842
2	0.683	-0.165	0.835
3	0.679	-0.168	0.832
5	0.663	-0.178	0.822
7	0.648	-0.188	0.812
9	0.634	-0.198	0.802
11	0.615	-0.211	0.789
13	0.591	-0.228	0.772
16	0.568	-0.245	0.755
19	0.535	-0.272	0.728
23	0.494	-0.306	0.694



K_{abs} = slope x 2.303

Slope = 0.00448 x 10^{-5}



CONCLUSION

Oxidation studies of thymidine in presence of various [Caffeic acid] and peroxydisulphate have been carried out under different experimental conditions. From competition kinetic studies of thymidine and caffeic acid for $PO_4^{\bullet -}$ percentage of protection of thymidine from for $PO_4^{\bullet -}$ with caffeic acid has been calculated. From the experimental quantum yield values (φexptl) and the calculated quantum yield values (φcal) assuming that caffeic acid acts as a scavenger of for $PO_4^{\bullet -}$, the percentage repair of thymine radicals is found to be 45%.

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