ijRASET

INTERNATIONAL JOURNAL
FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

www.ijraset.com

Call: ◎08813907089 | E-mail ID: ijraset@gmail.com

Effect of certain Additives on the Degradation of Chlorophyll-A and Pheophytin-A in Aqueous KOH and Acetonitrile Media: A Kinetic Study

T. Venugopal Rao[1], S. Venkata Raju[2], K. C. Rajanna[3], I. E. Chakravarthy[4]

[1, 4] Department of Chemistry, Rayalaseema University, Kurnool-518 007 (A.P.) India

[2, 3] Department of Chemistry, Osmania University, Hyderabad-500007 (T.S.) India

[1] Sri Chaitanya Post Graduate College, Mahaboobnaagar-509002 (T.S.) India

Abstract: Degradation of Chlorophyll-a (Chl-a) and Pheophytin-a (Pheo-a) followed first order kinetics in aqueous acetonitrile media using different compositions of water and acetonitrile in basic (KOH) media. Additives like ethanolamine (mono ethanol amine -MEA), diethanolamine (DEA), triethanolamine (TEA), isopropyl myristate (IPM), epichlorohydrin (ECH), and butylated hydroxy toluene (BHT) exhibited significant influence on the degradation constant. Benesi-Hildebrand (BH) and Rose- Drago (RD) methods were applied for the computation of Formation constants, molar extinction coefficient and ΔG values
Keywords: Degradation of Chlorophyll-a and Pheophytin-a; Effect of additives; Aqueous acetonitrile; Kinetics

I. INTRODUCTION

Chlorophylls are the few of the most important pigments present in green plants, algae, and several other microorganisms. Chlorophylls belong to class of pyrrole ring compounds, commonly known as porphyrins. Chlorophyll is a greenish pigment, which contains a porphyrin ring coordinated to central Mg atom. The structure is by and large similar to the heme-group found in hemoglobin except that heme contains Fe –atom at the center, while chlorophyll contains Mg. Chlorophylls are of two types, which are found in green plants, green vegetables in the form of chlorophyll-a and chlorophyll-b [1-3]. Chlorophyll a and chlorophyll b are similar in the main structure, with different substituents at the C-3 carbon position, as shown in figure-1 (Chlorophyll a has a methyl group whereas chlorophyll b has a formyl group).

Chlorophyll *a*, R = CH₃
Chlorophyll *b*, R = CHO
The porphyrin ring is shown in Red
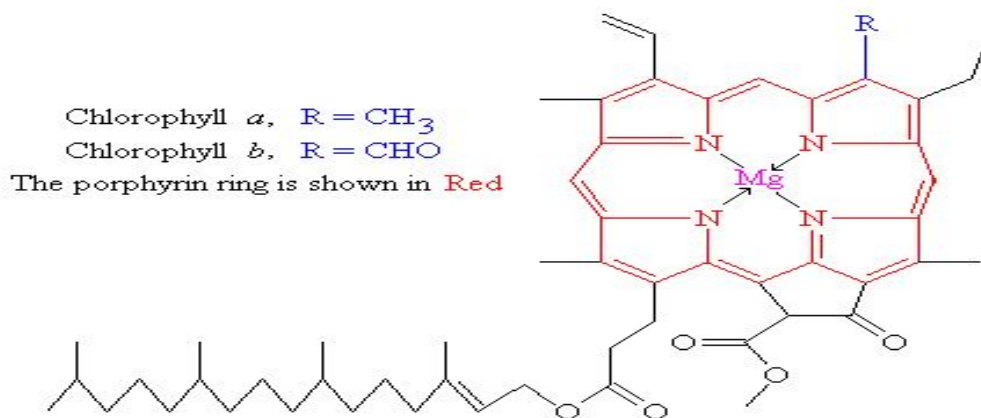
Fig –1. The structure of Chlorophyll-a and Pheophytin-a

In a recent review Marangoni summarized [4] the conversion of Chlorophyll-a into Pheophorbide-a occurs through two different processes. First path involves the treatment of Chlorophyll-a with acid, which removes the magnesium ion (Mg(II) by replacing with two hydrogen atoms. The resultant compound is Pheophytin-a, an olive-brown solid. Pheophytin-a thus obtained in turn upon hydrolysis, gives Pheophorbide-a. On the other hand, Chlorophyll-a upon enzymatic hydrolysis affords Chlorophyllide-a, which could be further degradaded to Pheophorbide-a as shown in Scheme-1.
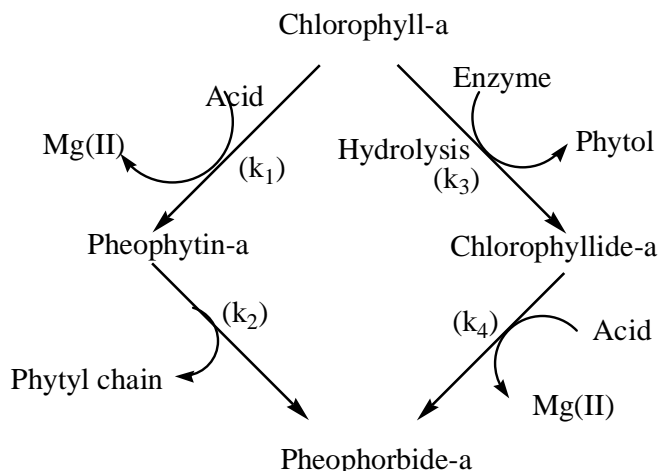
Scheme-1: Kinetic model for the conversion of Chlorophyll-a to Pheophorbide-a
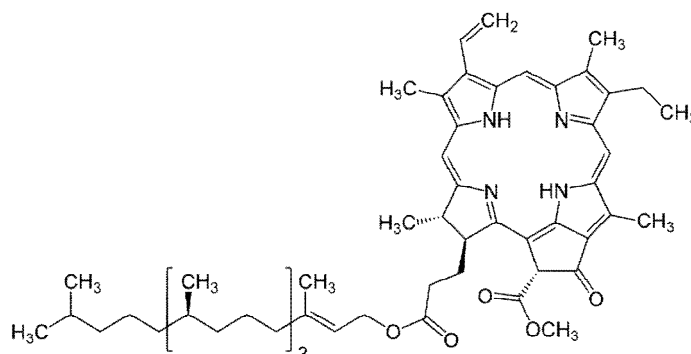

Fig-2: Structure of Pheophytin a (i.e. chlorophyll a without the Mg^{2+} ion)

Kinetic studies pertaining to the degradation of chlorophylls and their first stage products, have been the subject of interest to several food and agriculture chemists owing to the importance of green color stability of chlorophyll [4- 10]. In this part of the work, we want to explore the effect of food additives and stabilizers on the degradation of chlorophylls. Commonly used additives like ethanolamine (mono ethanol amine -MEA), diethanolamine (DEA), triethanolamine (TEA), isopropyl myristate (IPM), Epichlorohydrin (ECH), and butylated hydroxy toluene (BHT) have been used in present kinetic and interaction studies.

II. EXPERIMENTAL DETAILS

1) *Materials:* The AR grade chemicals are used through out the study. Chlorophyll-a from spinach (Aldrich, Bombay, India), Potassium hydroxide (KOH) (Merck, New Delhi, India), HPLC grade acetonitrile (Merck, New Delhi, India), hydrochloric acid (HCl), ethanolamine (mono ethanol amine -MEA), diethanolamine (DEA), triethanolamine (TEA), Isopropyl myristate (IPM), epichlorohydrin (ECH) and butylated hydroxy toluene (BHT) were procured from either SD fine Chemicals (India) or E-Merck (India). Pheophytin-a has been prepared from Chlorophyll-a according to standard literature procedures [11].

2) *Preparation of Pheophytin-a:* The chlorophylls were extracted from 50 gm of fresh spinach by blending with 100 mL of acetone. Celite was added, and filtered the mixture through Whatman filter papers (No.1 and No. 42) using a Buchner funnel. The residue was washed with 50 mL acetone, and the filtrate was transferred to a separating funnel. One hundred mL ethyl ether and 100 mL distilled water were added to produce a biphasic system. The ether layer was separated and acidified with 5 mL of aqueous HCl (1:3 HCl/Water v/v) to produce Pheophytins. The Pheophytin thus produced were washed several times with water, and the final ether solution was dried with granular sodium sulphate, filtered, and evaporated to dryness under vacuum. Pigments were redissolved in 12 mL of acetone, filtered through a 0.2 µm filter, and stored at -15 $^\circ$C. Stock solutions of Chlorophyll-a (1×10^{-5} mol/ dm^3) and Pheophytin-a (1×10^{-5} mol/ dm^3) were prepared in HPLC grade acetonitrile, while decimolar potassium hydroxide, emulsifiers and stabilizers were prepared in doubly distilled water.

3) *Kinetic Method:* Requisite amount of additive (emulsifier/ stabilizer/ potassium hydroxide), and acetonitrile solution of Chl-a / Pheo-a were taken in a volumetric flask, and thermostated at desired temperature. Progress of degradation of [Chl-a] (or [Pheo-a]) was monitored by measuring the absorbance value at 430 and 660 nm for Chl-a (and 410 and 670 nm for Pheo-a) at constant intervals of time on double beam Shimadzu (Chiyoda-ku, Tokyo, Japan) UV-VIS Spectrophotometer equipped with temperature control using 1-cm path length quartz curettes. Reactions were studied under pseudo first order conditions by taking [Additive] >> [Chl-a] (or [Pheo-a]. The pseudo-first-order rate constants were determined from the slopes of log (A_t) vs. time plots, where (A_t) is the absorbance (or optical density (OD)) corresponding to undegraded Chl-a (or Pheo-a) at any instant of time (t) depending on the system. The kinetic reactions were also carried out in different acetonitrile-water mixtures. The additives used in this reaction are ethanolamine (MEA), diethanolamine (DEA), triethanolamine (TEA), isopropyl myristate (IPM), Epichlorohydrin (ECH), and butylated hydroxy toluene (BHT) dissolved in binary solvent mixture of acetonitrile and water.

III. RESULTS AND DISCUSSION

1) *Kinetics and Mechanism of Hydrolysis of Chlorophyll-a in aqueous Acetonitrile Media:* The base catalyzed hydrolysis of Chl-a was studied using different initial [Chl-a] ranging from (0.90- 7.0) x10^{-6} M varying concentration of base (NaOH). From the rate data presented in table -1, it appears that apart from the added concentration of base plays a major role for hydrolysis. These results point out an irregular trend of rate dependence on the acetonitrile concentration. However, it is interesting to note that when the percentage of acetonitrile is increased from 10 to 95% the rate of hydrolysis shoots up significantly pointing out that removal of Mg (II) is more favorable in low dielectric medium. This could be probably due to the complexation Mg (II) with acetonitrile.

Table-1: Effect of variation of at Acetonitrile (%) on the Rate of hydrolysis of Chlorophyll-A in basic media

% ACN	Rate constant 10^3k' (hr^{-1}) and [KOH] at 30^{0}C					Rate of hydrolysis with [KOH]
	0.010	0.020	0.030	0.040	0.050	
10	3.52	3.52	3.52	3.52	3.52	Constant
25	3.36	3.36	3.36	3.36	3.36	Constant
50	5.53	3.08	2.84	2.84	2.84	Decreased and remained constant
75	3.88	5.13	6.46	7.48	9.1	Increased
95	10.9	11.9	12.6	13.2	14.1	Increased

Data presented in table-3 points out that rate of hydrolysis (k'/hr)) is almost doubled with a 10 degrees rise in temperature from 15^{0}C to 25^{0}C showing the applicability of Arrhenius theory of activation energy (E_a). Enthalpy of activation ($\Delta H^\#$) is computed from the relationship,

$$\Delta H^\# = E_a - RT$$

Free energy of activation is computed using Eyring's equation,

$$\Delta G^\# = RT \ln (RT/Nhk')$$

Enthalpy of activation is obtained from one of the form of Gibbs-Helmholtz equation,

$$\Delta S^\# = (\Delta H^\# - \Delta G^\#)/ T$$

Substitution of respective values afforded the energy of activation (Ea) = 91.3kJmol^{-1}, enthalpy of activation ($\Delta H^\#$) = 88.8 kJ mol^{-1}, free energy of activation ($\Delta G^\#$) = 86.7 kJmol^{-1}, and entropy of activation ($\Delta S^\#$) = 7.05 JK^{-1} mol^{-1}. Small positive entropy of activation ($\Delta S^\#$) obtained probably points out the transition state due to solvation.

2) *Kinetics and Mechanism of Hydrolysis of Pheophytin-a in Aqueous Acetonitrile Media: It* is well known that hydrolysis of Pheophytin-a (reverse of esterification) splits off phytol and gives Pheophorbide-a. The kinetics of hydrolysis of Pheophytin-a was also studied in various compositions of water-acetonitrile solutions (10% to 95% acetonitrile) in acidic as well as basic media (25% acetonitrile). It is interesting to note that hydrolysis of Pheophytin is not favorable in basic medium even at elevated temperatures. This observation may probably point out that activation of Pheophytin is highly sensitive to temperature.

T

able-5: Kinetics of Chlorophyll-a and Pheophytin-a hydrolysis in the presence of Some Additives

S. No	Additive	Rate of hydrolysis of Chlorophyll-a	Rate of hydrolysis of Pheophytin-a
1	MEA	Marginal increase	Gradual decreases
2	DEA	Almost constant	Gradual increases
3	TEA	A gradual increase with a limiting value at higher [TEA]	Almost constant
4	IPM	Gradually increases	Gradual decreases
5	ECH	A gradual increase with a limiting value at higher [ECH]	A gradual increase with a limiting value at higher [ECH]
6	BHT	A gradual increase with a limiting value at higher [BHT]	A gradual increase with a limiting value at higher [BHT]

IV. CONCLUSIONS

A. Spectrophotometric observations of Chlorophyll-a as well as Pheophytin-a did not indicate CT bands. Formation of molecular complexes were ascertained from either hyper chromic, or hypso chromic shifts in the case of additives.

B. Formation constant and molar extinction coefficient values were calculated in all interaction studies with the help of Benesi-Hildebrand method and the same values were confirmed by Rose - Drago method.

C. Kinetic study of hydrolysis of either Chlorophyll-a or Pheophytin-a is sensitive and depends on the medium.

D. Hydrolysis of Chlorophyll-a and Pheophytin-a are by and large complementary to each other.

V. ACKNOWLEDGEMENTS

REFERENCES

[1] Streitwieser (Jr),A.; Heathcock, C.H. Introduction to Organic Chemistry, (MacMillan, New York, 1981).

[2] Stryer, L. Biochemistry, (W.H. Freeman and Co, San Francisco, 1975).

[3] Belitz, H. D.; Grosch, W.; Schieberle, P. Food Chemistry. 4th ed. Verlag Berlin Heidelberg: Springer. (2009), 1070

[4] Marangoni, A. G. Kinetic Analysis of Food Systems (Chapter-2) 2017, 55-63.

[5] Heaton, J.W.; Marangoni, A.G., Trends. Food. Sci.Technol., 1996, 7, 8–15

[6] Heaton, J.W.; Yada, R.Y.; Marangoni, A.G. J .Agric. Food. Chem. 1996, 44, 395–398

[7] Heaton, J.W.; Lencki, R.W.; Marangoni, A.G. J .Agric. Food. Chem.1996, 44, 399–402

[8] Minguez-Mosquera, M.I.; Garrido-Fernandez, J.; Gandul-Rojas, B. J .Agric. Food. Chem.1989, 37, 8–11

[9] Van Boekel M.A.J.S. Anniversary Review: kinetic modeling in food science: a case study on chlorophyll degradation in olives. J .Agric. Food. Chem., 2000, 80, 3–9

[10] White, R.C.; Jones, I.D.; Gibbs, E. J. Food. Sci., 1963, 28, 431–435

[11] Vernon, L. Anal. Chem., 1960,32,1143-1150

[12] Laidler, K.: J. Chemical Kinetics, Pearson Education, Singapore (2004)

[13] Glasstone, S., Laidler, K. J., Eyring, H.: "Theory of Rate Processes", McGraw Hill & Co., New York (1961)

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089 ☷ (24*7 Support on Whatsapp)