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Bisulfate and Boric Acid Effects on Chlorophyll Degradation Kinetics

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Abstract: The authors have studied the effect of sodium bisulfate (NaHSO₄), and boric acid (BA) on degradation of Chlorophyll (Chl) in (5% V/V) aqueous acetonitrile medium at various temperatures (in the range:300-360 K). Boric acid did not affect the degradation of Chlorophyll even after two three days, while sodium bisulfate (NaHSO₄) revealed significant effect on the degradation to pheophytin. The reaction obeyed first-order kinetics in [Chpl], under the conditions [NaHSO₄] >> [Chpl]. Variation of added [NaHSO₄], and obeyed the rate law: $(k') = k_u + k_{cat} [NaHSO_4]$. Large negative entropy of activation ($-\Delta S^\#$) values in general might be attributed to the rigid nature of transition state due to a greeter solvation bulkier chlorophyll. In addition to this, the entropy of activation ($\Delta S^\#$) for uncatalysed reaction is relatively more negative than bisulfate catalysed reaction, while the enthalpy of activation ($\Delta H^\#$) for catalysed reaction is less than that of uncatalysed reaction. This trend probably accounts for greater random ness of molecules followed by more activation collisions in the catalysed system.
Keywords: sodium bisulfate; boric acid; degradation kinetics; Chlorophyll; pheophytin

I. INTRODUCTION

Chlorophyll is a naturally important metal chelate, which exists as the green pigment of plants. In chlorophyll the central metal ion is magnesium (Mg) that is bonded to four porphyrin molecules through nitrogen atoms in a square planar arrangement. The absorption peaks of Chlorophyll a are at 665 nm and 430 nm. Chlorophyll a fluoresces at 673 nm.

During the past several years several workers studied the kinetics of Chlorophyll (Chl) degradation extensively [1-10], and proposed different pathways for its degradation. In 1941, Mackinney and Joslyn [1] reported that the rate of Chlorophyll degradation followed a first-order kinetics, while Lajollo et al [2] reported that degradation of chlorophyll-a occurred 2.5 times faster than chlorophyll-b degradation at 37°C, and 0.32 water activity (a_w). Schwartz and Lorenzo (1991) showed that during storage of aseptically processed spinach puree, Chl- a form degraded 4 to 10 times faster than the Chl-b form [3]. During their extensive studies on the degradation kinetics of Chlorophylls and Chlorophyllides Canjura and co-workers stated that that Chl-a is much more susceptible for degradation than Chl-b [4]. Degradation models proposed by Heaton [5], White [6], and Minguez- Mosquera [7] are also noteworthy among other kinetic models for Chl degradation. In their publication Heaton's research group developed a kinetic and mechanistic model to explain the degradation of chlorophyll to Pheophytin, chlorophyllide, and pheophorbide in coleslaw, pickles, and olives [5]. Kinetic constants for each step was evaluated in their model. The work of Gupte et al [8] revealed that degradation kinetics of chlorophyll obtained from spinach puree obeys first order kinetics. Schwartz and Elbe [9] took up elaborate kinetics studies and developed a kinetic model for the degradation of chlorophyll to Pheophytin (Pheop), which in turn afforded

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pyropheophytin (Py-Pheop). Alejandro Marangoni's research group developed a general kinetic and mechanistic model to explain chlorophyll degradation to chlorophyllide, pheophytin, and pheophorbide in pickles and olives [10].

In recent past there has been enhanced interest in the exploration of sodium bisulfate (NaHSO$_4$ or SBS) [11-13] and boric acid (BA) [14-17] as highly reactive catalysts in organic synthesis because they are economically cheap, environmentally safe, easily available, eco-friendly easy to handle during work-up followed by affording the corresponding products in excellent yields with high selectivity. Enthused by the effect of several additives on the degradation, coupled with a broad spectrum of utilities of boric acid (BA), we herein would like to report the effect of BA on the degradation kinetics of Chlorophyll in aqueous medium.

II. EXPERIMENTAL

Materials: All the chemicals were purchased from Avra, Aldrich or Arcos Organics and used without further purification. Chlorophyll-a was always prepared fresh in our laboratory from spinach leaves according to Strain and Svec [18].

A. Kinetic Method of Following the Reaction

Reaction flask containing required amount of additive (boric acid (BA) or NaHSO$_4$) suitable solvent (generally aqueous acetonitrile solvent) and another flask containing the Chlorophyll was clamped in a thermostat (constant temperature bath) at a desired temperature to attain thermal equilibrium. Contents of both the reaction flasks are mixed instantaneously and mixed thoroughly to initiate the reaction. Due care is taken by coating the flasks from outside with a Japan black to prevent photochemical effects. Aliquots of the reaction mixture were withdrawn into a cuvette and placed in the cell compartment of the laboratory visible spectrophotometer. Cell compartment was provided with an inlet and an outlet for circulation of thermostatic liquid to maintain a desired temperature. Absorbance (A) of the reaction mixture (Chlorophyll) was noted down at various time intervals at 430 nm. Absorbance values were in agreement with each other with an accuracy of ± 3 percentage error.

Determination of the Order of Reaction: In the present kinetic protocol, we have considered the absorbance of Chlorophyll (Chl) before degradation as (A$_0$), while (A$_t$) as the absorbance during the course of reaction at a given time is (t). To determine **"Order of the reaction"**, we have used graphical method of approach, based on the integrated rate expressions given for the first order reactions in literature [23]. Under the conditions, viz., [Additive]$_0$ >> [Chl]$_0$ the plots of ln [(A$_0$)/ (A$_t$)] versus time were linear with positive slope passing through origin (figure- 1), suggesting first order kinetics with respect to [Chl], which is in accordance with the following equation:

Fig.1 -Plots of ln(A$_0$/A$_t$) vs Time in aq.NaHSO$_4$ media at 323 K

Set-1; y = 0.0036x; R^2 = 0.981
Set-2: y = 0.0041x' R^2 = 0.986
Set-3: y = 0.0042x' R^2 = 0.988
Set-4: y = 0.0044x; R^2 = 0.976
Set-5; y = 0.005x; R^2 = 0.962
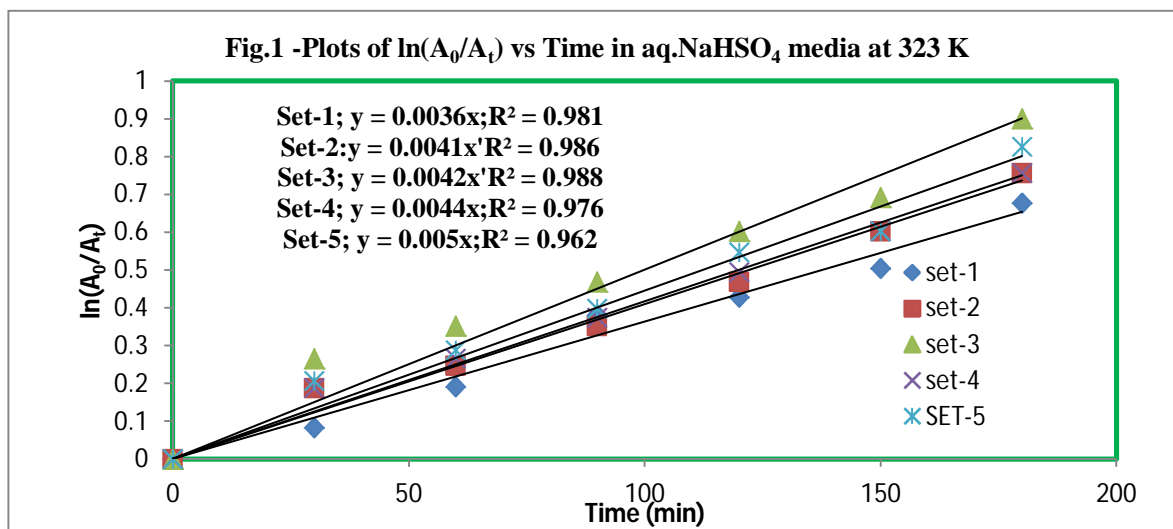
$$ln\left[(A_0)/(A_t)\right] = k't \qquad (1)$$

Fig.1 -Plots of ln(A$_0$/A$_t$) vs Time in aq. NaHSO$_4$ media at 323 K

10^6[Chpl]=5.0mol/dm^3 ; Solvent =
10^3[NaHSO$_4$] = 1.00 (set-1),2.00 (set-2);3.00(set-3); 4.00 (set-4); 5.00 (set-5) mol/dm^3

Slope of this linear plot afforded pseudo first order rate constant (k'). When these experiments conducted at different the initial concentration of [Additive] at fixed concentrations of Chlorophyll (Chl), and temperature, pseudo first order rate constants (k')

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increased with [NaHSO$_4$], as shown in Fig-1. The plots of (k'), values against [NaHSO$_4$], gave straight line (Figure 2) with a positive slope and intercept on ordinate showing that experimentally observed degradation rate of Chlorophyll is the algebraic sum of spontaneous and acid dependent degradations of Chlorophyll, as shown in the following equation:

$$(k') = k_u + k_{cat} [NaHSO_4] \qquad (2)$$

The values of (k'_u) *and* (k'_{cat}) were obtained from the slope and intercept values, which increased considerably with an increase in temperature.


Fig.2 - Plot of k' Vs 10³ [NaHSO₄] at 357 K

y = 0.0015x + 0.0015; R² = 0.9941
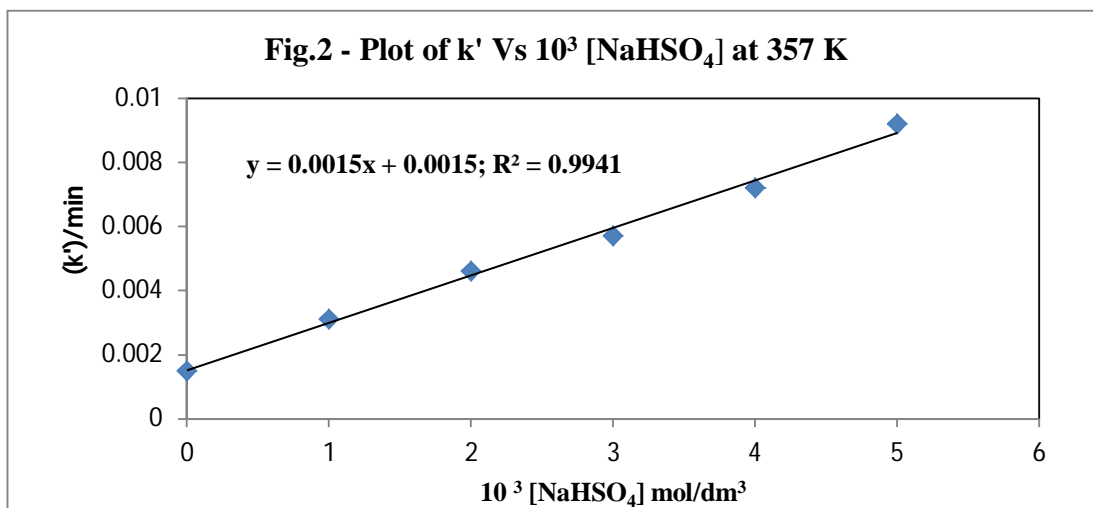
Fig.2 – Effect of Sodium bisulphate on Chlorophyll degradation Plot of (k') versus 10^3[NaHSO$_4$] at 357K (10^6[Chpl]=5.0 mol/dm^3)

Free energy of activation ($\Delta G^{\#}$) can be obtained using rate constant (k) at any given temperature from the following equation according to Eyring's theory of reaction rates [19,20],

$$k = (k_t) (RT/Nh) \exp(-\Delta G^{\#}/RT) \qquad \ldots (3)$$

Where the transmission coefficient (k_t) is equal to unity, R, N, h and T represent the gas constant, Avogadro number, Planck's constant and reaction temperature; and $\Delta G^{\#}$ represent free energy of activation respectively. After appropriate rearrangement, a natural logarithmic form of this equation comes out as,

$$\Delta G^{\#} = RT \ln (RT/Nhk) \qquad \ldots (4)$$

Substituting the values for R (8.314 J/ mol/K), N (6.022 x 10^{23} /mol), h (6.626 x 10^{-34} J s) and T (Kelvin degrees) respectively. Substituting for R, N, and h (in SI units), $\Delta G^{\#}$ could be simplified accordingly into equation (5) as given below,

$$\Delta G^{\#} = 8.314 \times T[23.7641 + \ln(T/k)] \qquad \ldots (5)$$

The free energy of activation ($\Delta G^{\#}$) has been evaluated at various temperatures and used for the evaluation of enthalpy and entropies of activation ($\Delta H^{\#}$ and $\Delta S^{\#}$), using Gibbs – Helmholtz equation (eq-6):

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#} \qquad \ldots (6)$$

B. Mechanism of Degradation of Chlorophyll

In aqueous medium bisulfate dissociates to release proton (H$^+$) and (SO$_4^{2-}$) as shown in the following dissociation step,

$$HSO_4^- \underset{}{\overset{K_d}{\rightleftharpoons}} H^+ + SO_4^{2-} \quad (7) \quad K_d = [H^+][SO_4^{2-}] / [HSO_4^-]$$

$$Chlorophyll \xrightarrow{Slow\ (k_1)} Products \quad (8)$$

$$Chlorophyll + H^+ \xrightarrow{Slow\ (k_2)} Products \quad (9)$$
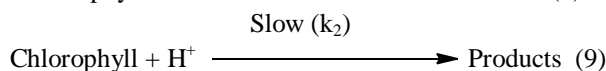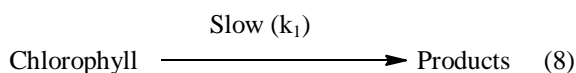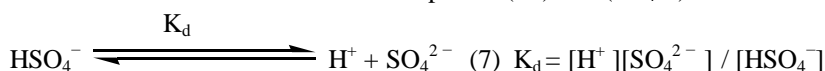
Rate law for the sequence of steps could be given as,

(Rate) = - d [Chpl] /dt = k_1 [Chpl] + k_2 [Chpl] [H$^+$]

This equation could be rearranged as,

(Rate) = - d [Chpl] /dt = (k_1 + k_2 [H$^+$]) [Chpl]

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This equation could be further simplified as,

(Rate)/ [Chpl] = (- d [Chpl] /dt) / [Chpl] = (k') = $k_1 + k_2$ [H$^+$]

But from the dissociation of Bisulfate (HSO$_4^-$) step (6), [H$^+$] could be written as,

[H$^+$] = K_d [HSO$_4^-$] /[SO$_4^{2-}$]

Substituting for [H$^+$] in above equation (k') comes out as,

$$(k') = k_1 + k_2 K_d [HSO_4^-] /[SO_4^{2-}] \quad ..... (10)$$

This equation is similar to equation (2), and $k_u = k_1$; $k_{cat} = (k_2 K_d /[SO_4^{2-}])$,

In the present study, k_u *and* k_{cat} were evaluated at different temperatures and used for the calculation of activation parameters.
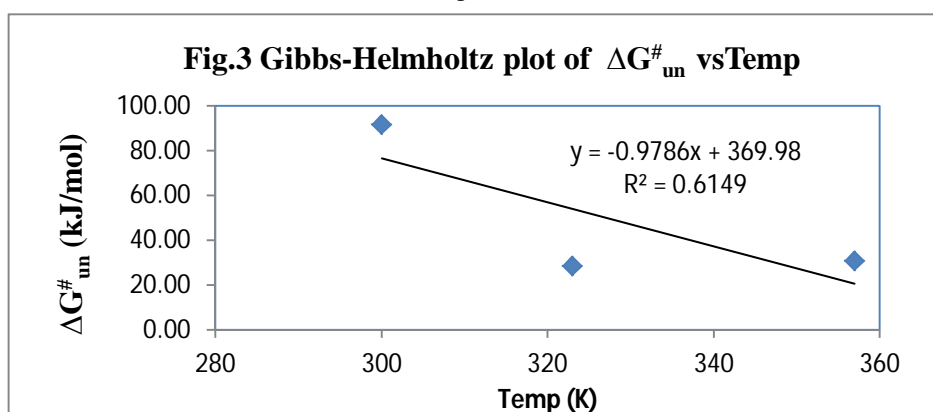

Fig.3. – Gibbs-Helmholtz plot of $\Delta G^\#_{un}$ vsTemp for the degradation of Chlorophyll
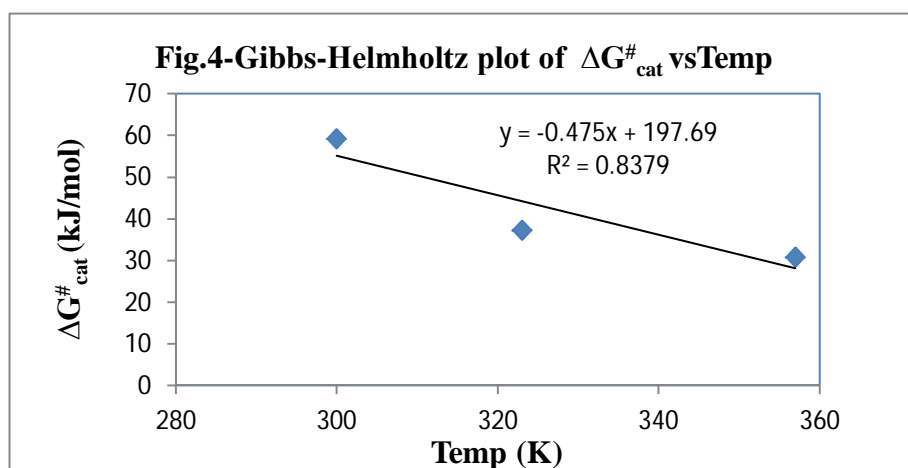
Fig- 4: Gibbs-Helmholtz plot of $\Delta G^\#_{cat}$ vsTemp for the degradation of Chlorophyll

From the Gibbs-Helmholtz plots (Figures 3 and 4), the activation enthalpy and entropy were obtained and compile below. Large negative entropy values in general might be attributed to the rigid nature of transition state due to a greeter solvation bulkier chlorophyll. In spite of this, the entropy of activation for uncatalysed reaction is relatively more negative than bisulfate catalysed reaction, while the enthalpy of activation for catalysed reaction is less than that of uncatalysed reaction. This trend probably accounts for greater random ness of molecules followed by more activation collisions in the catalysed system.

Activation parameter	Uncatalysed degradation	Bisulfate degradation
$\Delta H^\#$ (kJ/mol)	370	198
$-\Delta S^\#$ (J/K/mol)	979	475

When we tried to explore the effect of the other greenery additive boric acid (BA) in chlorophyll degradation, we have noticed that degradation did not occur even at higher temperatures, and the chlorophyll solution remained greenery for two to three days. It

appeared that boric acid (BA) stabilized the greenery of chlorophyll and did not hydrolyze or degrade in the presence of boric acid. Haisman and Clarke [21] earlier reported that addition of the NaCl stabilized chlorophylls and the green color of food products although the mechanism is not unequivocally established. Recently the work of Nisha et al [22] revealed that the addition of NaCl (1% and 2%) added to spinach puree improved the stabilization of green color even it is heated at 50 to 120 °C.

III. CONCLUSIONS

Kinetics of degradation of chlorophyll to pheophytin revealed first-order kinetics in [Chpl], under the conditions [NaHSO$_4$] >> [Chpl], while variation of added [NaHSO$_4$] obeyed the rate law: $(k') = k_u + k_{cat} [NaHSO_4]$. Observed negative entropy of activation $(-\Delta S^{\#})$ values in general might be attributed to the rigid nature of transition state due to a greeter solvation bulkier chlorophyll. In addition, the entropy of activation $(-\Delta S^{\#})$ for uncatalysed reaction is relatively more negative than bisulfate catalysed reaction, while the enthalpy of activation $(\Delta H^{\#})$ for catalysed reaction is less than that of uncatalysed reaction. However, added boric acid did not affect the degradation of Chlorophyll even after two three days indicating the stabilization of greenery of Chlorophyll.

IV. ACKNOWLEDGMENTS

A. Statement for Conflict Interest

Authors declare that they do not have any conflict of interest.

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