ENTHALPY-ENTROPY COMPENSATION IN FOOD REACTIONS

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By

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Approval of the Graduate School of Natural and Applied Sciences.

for Prof.Dr. Alpay ANKARA

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To my family

ABSTRACT

ENTHALPY-ENTROPY COMPENSATION IN FOOD REACTIONS

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M.Sc. in Food Engineering

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The loss of ascorbic acid in aqueous solution and in orange juice due to changes in temperature and pH was determined by HPLC in order to analyze the presence of enthalpy-entropy compensation effect.

Assuming a first-order kinetics, the rate constants for ascorbic acid loss were calculated by linear least squares analysis of data in logarithm of concentration versus time plots at each different temperature and pH conditions. Kinetic parameters of ascorbic acid loss were calculated from the Arrhenius plots. Experimental activation energies for ascorbic acid loss were in the range of 18.5 and 36.4 kJ/mol between pH 5.82 and 2.38 for aqueous solution and in the range of 15.8 and 99.7 kJ/mol between pH 5.05 and 2.05 for orange juice.

The values of activation enthalpy and activation entropy were determined from the slope and from the intercept of

Arrhenius plots at harmonic mean of experimental temperatures. For the loss of ascorbic acid in both aqueous solutions and in orange juice, excellent linear plots of enthalpy-entropy data pairs were obtained. The isokinetic temperature, which was obtained from the slope of activation enthalpy-activation entropy plots was found to be 291.1 K for ascorbic acid loss in orange juice and 341.2 K for ascorbic acid loss in aqueous solutions.

In order to understand whether the compensation is the result of statistical effects which arise due to experimental errors or is true chemical compensation, several test methods suggested by many authors were applied to the data of present work. According to the results of these testing procedures, it was concluded that true chemical compensation phenomena was observed in both orange juice and aqueous solution for the loss of ascorbic acid.

Key words : Enthalpy-entropy compensation, isokinetic temperature, kinetics of ascorbic acid loss, ascorbic acid in orange juice.

ÖZET

GIDA REAKSİYONLARINDA ENTALPİ-ENTROPİ DENKLEMESİ

İZGİ, Esra

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Tez Yöneticisi : Doç.Dr. Güler AYRANCI

Şubat 1989 , 90 sayfa

Entalpi-entropi denklemesinin varlığının analizi için portakal suyunda ve sulu solisyonda sıcaklık ve pH değişimiyle askorbik asit kaybı Yüksek Basınçlı Sıvı Kromatografisi kullanılarak yapıldı.

Birinci derece reaksiyon kinetiği varsayımıyla, herbir değişik pH ve sıcaklık ortamında reaksiyon hızı sabitleri en küçük kareler doğrusu metodu kullanılarak konsantrasyonun logaritmasının zamana karşı çizilmiş grafiklerinden hesaplandı. Askorbik asit kaybı için kinetik parametreler Arrhenius grafiklerinden bulundu. Askorbik asit kaybı için deneysel etkinleştirme enerjileri sulu solisyonlarda pH 5.82 ile 2.38 arasında 18.5-36.4 kJ/mol, portakal suyunda pH:5.05-2.05 arasında 15.8 ve 99.7 kJ/mol. olarak bulundu.

Etkinleştirme entalpisi ve etkinleştirme entropisi deneysel sıcaklıkların uyumlu ortalamasında Arrhenius

çizimlerinin eğiminden ve kesim noktasından bulundu. Portakal suyundaki ve sulu solisyondaki her iki askorbik asit kaybı için entalpi-entropi veri çiftlerinden mükemmel doğrusal grafikler elde edildi. Bu grafiklerin eğiminden bulunan isokinetik sıcaklık portakal suyundaki kayıp için 291.1 K ve sulu solisyonlar için 341.2 K olarak hesaplandı.

Denklemin deneysel hatalardan kaynaklanan istatistiki olayların sonucu veya doğru kimyasal denkleme olup olmadığını anlayabilmek için bazı araştırmacılarca önerilen bir seri test metodları uygulandı. Bu test metodlarına göre portakal suyunda ve sulu solisyonlarda gözlenen denklemenin doğru kimyasal denkleme olduğu sonucuna varıldı.

Anahtar kelimeler : Entalpi-entropi denklemesi, isokinetik sıcaklık, askorbik asit kaybı kinetiği, portakal suyundaki askorbik asit.

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CHAPTER I

INTRODUCTION

I.1. Thermodynamic Formulation of Reaction Rates

The rate of any chemical reaction may be expressed as the change in the concentration of a reactant or of a product with time.

The effect of varying temperature has been the most important key to the theory of rate processes. In the last century, many famous thermodynamicists, such as van't Hoff in 1884,Hood in 1885, and Arrhenius in 1889, were concerned with explaining the effects of temperature on reaction rates.

In 1889, Arrhenius pointed out that since the van't Hoff equation for the variation of the equilibrium constant was

$$\frac{d\ell n\, K_c}{dT} = \frac{\Delta E}{RT^2} \qquad (1)$$

where K_c is the equilibrium constant expressed in terms of concentrations, and ΔE is the energy change, for a chemical

reaction of the type A + B $\rightleftharpoons$ C + D, the equilibrium condition can be formulated by equating the rates of the two opposing reactions ;

$$k_1 [A][B] = k_{-1} [C][D] \qquad (2)$$

where k_1 and k_{-1} are the second order rate constants for the reaction. The equilibrium constant is equal to k_1/k_{-1}

$$K_c = \frac{[C][D]}{[A][B]} = \frac{k_1}{k_{-1}} \qquad (3)$$

The van't Hoff equation (Equation 1) can now be written as ;

$$\frac{d \ell n\, k_1}{dT} - \frac{d \ell n\, k_{-1}}{dT} = \frac{\Delta E}{RT^2} \qquad (4)$$

which may be split into the two equations ;

$$\frac{d \ell n\, k_1}{dT} = \frac{E_1}{RT^2} + I \qquad (5)$$

$$\frac{d \ell n\, k_{-1}}{dT} = \frac{E_{-1}}{RT^2} + I \qquad (6)$$

where $E_1 - E_{-1} = \Delta E$. Experimentally it was found that I can be set equal to zero, the rate constant therefore being related to the temperature by an equation of the form ;

$$\frac{d\ell n\, k}{dT} = \frac{E}{RT^2} \qquad (7)$$

With this result, one can assume that the reaction path from A+B to C+D can be divided into two parts, one of which is concerned with the rate from left to right, the other from right to left. The rate of reaction from left to right is controlled by the energy E_1 and that of reaction from right to left is controlled by the energy E_{-1} as represented in Figure 1.

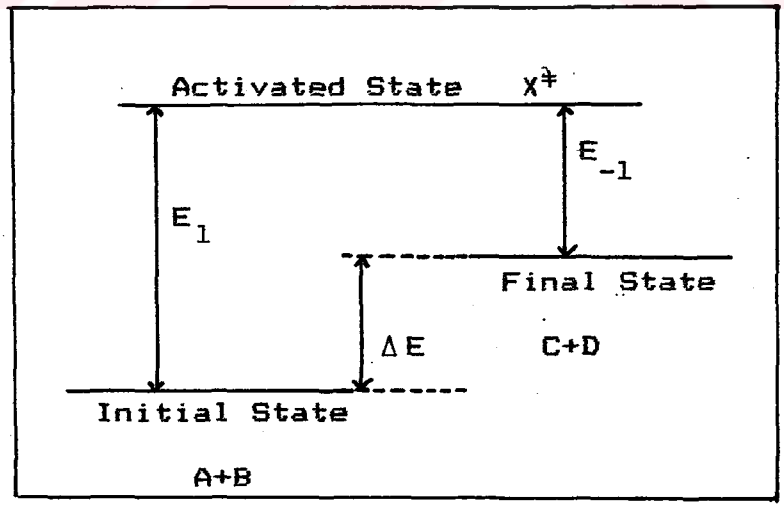


Figure 1. Schematic energy diagram, showing the relative energies of the initial, final and activated states for a reaction A+B $\rightleftarrows$ C+D.

The intermediate state between the initial and final states is referred to as the "activated state" or the "transition state" as will be explained later in the discussion of the Transition State Theory of reaction rates. The energy E_1 is called as the energy of activation for the forward process and energy E_{-1} is called as the energy of activation for the backward process.

The Equation 7 integrates to

$$\ell nk = - \frac{E}{RT} + \text{constant} \qquad (8)$$

provided that E is independent of temperature. If the constant is taken as ℓnA, Equation 8 can be written as ;

$$k = A \exp(-E/RT) \qquad (9)$$

The Equation 9 is known as the Arrhenius equation where A is a constant usually referred to as the "frequency factor" or "preexponential factor". The exponential term exp(-E/RT) gives the fraction of the reactant molecules that have energies in excess of the value of E and is named as the Boltzmann expression.

When ℓnk values for a reaction are plotted with respect to the reciprocals of corresponding absolute temperatures, a

straight line should be obtained with the slope equal to -E/R according to Equation 9.

According to the Arrhenius law, in order to completely understand the factors determining the rate constant of a reaction, the terms "activation energy" and "frequency factor" should be well understood. Activation energies can be interpreted using the quantum mechanical methods such as potential energy surfaces [1].

Discussion of the quantitative theory of the frequency factor involves the Kinetic Theory of Collisions and the Transition State Theory.

In the Kinetic Theory of Collision, the molecules are treated as hard spheres making elastic collisions. For a bimolecular reaction between A and B, the rate constant is given by [2] ;

$$k = N\, d_{AB}^2 \left[8\pi\, \underline{k}\, T \frac{m_A + m_B}{m_A\, m_B} \right]^{1/2} \exp\,(-E/RT) \qquad (10)$$

where,

N = Avogadro number

d_{AB} = The sum of the radii of A and B = $r_A + r_B$

d_{AB}^2 = the collision cross-section

$\underline{k}$ = Boltzmann constant = 1.3806×10^{-23} JK^{-1}

$$\frac{m_A + m_B}{m_A\, m_B} = \frac{1}{\mu}$$, μ is the reduced mass.

When Equation 10 is compared with the Arrhenius equation $k = A \exp(-E/RT)$, it seems that the frequency factor A is equivalent to

$$A = N\, d_{AB}^2 \left[\frac{8 \pi \underline{k}\, T}{\mu}\right]^{1/2} \qquad (11)$$

which is known as the collision number per cm per sec. Therefore, the collision theory suggests that the rate is proportional to the square root of temperature.

$$k \propto T^{1/2} \exp(-E/RT) \qquad (12)$$

Transition-State Theory, on the other hand accepts that in order for any chemical change to take place, the atoms or molecules should come together to form a transition or activated complex. This complex is regarded as being situated at the top of an energy barrier lying between the initial and final states as shown in Figure 1. The rate of the reaction is assumed to be controlled by the rate with which the complex travels over the top of the barrier. The theory also involves the hypothesis that even when the reactants and

products are not at equilibrium with each other, there exists an equilibrium between the reactants and activated complexes.

For the reaction $A+B \rightleftharpoons X^{\ddagger} \rightleftharpoons C+D$, the equilibrium between the reactants and activated complexes may be written as;

$$\frac{[X^{\ddagger}]}{[A][B]} = K^{\ddagger} \qquad (13)$$

where $[X^{\ddagger}]$ refers to the concentration of activated complexes and $K^{\ddagger}$ is the equilibrium constant for activation.

The treatment of this equilibrium constant by statistical mechanics involves the partition functions of the reactants and activated complex [1] and it is out of the scope of this thesis. However, the relation between the rate constant of the reaction and $K^{\ddagger}$ can be given as ;

$$k = \frac{\underline{k}T}{h} K^{\ddagger} \qquad (14)$$

where $\underline{k}$ is the Boltzmann constant, 1.38×10^{-23} JK^{-1} and h is the Planck's constant 6.62×10^{-34} J.sec .

When $K^{\ddagger}$ is expressed in terms of the free energy of activation, $\Delta G^{\ddagger}$, Equation 14 can be written as ;

$$k = \frac{kT}{h} e^{-\Delta G^{\ddagger}/RT} \qquad (15)$$

Since

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \qquad (16)$$

where $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ represent the enthalpy of activation and entropy of activation respectively, Equation 15 may be written in the following way ;

$$k = \frac{kT}{h} e^{\Delta S^{\ddagger}/R} e^{-\Delta H^{\ddagger}/RT} \qquad (17)$$

This equation is the expression of rate constant in terms of the thermodynamic functions enthalpy and entropy. It is possible to convert the Equation 17 into one which involves the experimental activation energy, E_{exp} , instead of the enthalpy of activation , $\Delta H^{\ddagger}$.

Since $K^{\ddagger}$ is a concentration equilibrium constant, its variation with temperature can be written as ;

$$\frac{d\ell n K^{\ddagger}}{dT} = \frac{\Delta E^{\ddagger}}{RT^2} \qquad (18)$$

where $\Delta E^{\ddagger}$ is the increase in energy in passing from the

initial state to the activated state.

On the other hand, the differentiated form of the logarithm of Equation 14 is ;

$$\frac{d\ell n\ k}{dT} = \frac{1}{T} + \frac{d\ell n\ K^{\neq}}{dT} \qquad (19)$$

Replacement of Equation 18 into Equation 19 is ;

$$\frac{d\ell n\ k}{dT} = \frac{1}{T} + \frac{\Delta E^{\neq}}{RT^2} = \frac{RT + \Delta E^{\neq}}{RT^2} \qquad (20)$$

Since the experimental activation energy is obtained by plotting the logarithm of k against the reciprocal of the absolute temperature, the experimental activation energy, E_{exp}, is defined by ;

$$\frac{d\ell n\ k}{dT} = \frac{E_{exp}}{RT^2} \qquad (21)$$

Comparing the Equations 20 and 21, one can write ;

$$E_{exp} = \Delta E^{\neq} + RT \qquad (22)$$

Since

$$\Delta H^{\neq} = \Delta E^{\neq} + P\Delta V^{\neq} \qquad (23)$$

Equation 22 becomes ;

$$E_{exp} = \Delta H^{\neq} + RT - P\Delta V^{\neq} \qquad (24)$$

where $\Delta V^{\neq}$ is the change in the volume of the reaction mixture during activation.

Considering that $P\Delta V^{\neq} = \Delta n^{\neq}RT$ for gas reactions, where $\Delta n^{\neq}$ is the change in the number of molecules, when the activated complex is formed from the reactants, the rate constant expressions for the unimolecular and bimolecular reactions are given in Equations 25a and 25b.

$$k = e \frac{kT}{h} e^{\Delta S^{\neq}/R} \quad e^{-E_{exp}/RT} \qquad (25a)$$

$$k = e^{2} \frac{kT}{h} e^{\Delta S^{\neq}/R} \quad e^{-E_{exp}/RT} \qquad (25b)$$

For reactions in solution, $\Delta V^{\neq}$ is small, hence ;

$$E_{exp} = \Delta H^{\neq} + RT \qquad (26)$$

When Equation 25a is compared with the Arrhenius equation, $k = Ae^{-E_{exp}/RT}$, the term $e \frac{kT}{h} e^{\Delta S^{\neq}/R}$ gives the value of the frequency factor A for a unimolecular reaction.

If we compare these two reaction rate theories, in collision theory reaction rate depends on number of energetic collisions between reactants and intermediate breaks down

rapidly enough into product without influencing the rate of overall process. However, in transition state theory the reaction rate is governed by the rate of decomposition of intermediate. The rate of formation of intermediate is assumed to be so rapid that it is present in equilibrium concentrations at all times. The rate controlling step is the first step in collision theory, whereas, the second one in transition state theory.

I.2. Enthalpy-Entropy Compensation-Previous Studies

In the past 20 years, the study of reaction rates has led to the observation that there are a number of equilibria and rate processes in which plotting enthalpic changes against entropic changes produced by a systematic variation of solvent composition, reactant molecule change or pH gives a straight line. The slope of the line in such a graph which has the dimensions calories per mole over calories per mole degree, hence absolute temperature is designated as isokinetic temperature, T_c, and the relationship is called enthalpy-entropy compensation. If the enthalpies and entropies are sufficiently insensitive to temperature changes or if the required change in temperature is small, the isokinetic temperature will be a real temperature at which the variation in logk among set of correlated reactions is a minimum, if not zero.

Evidence for compensation effect occurs in phenomena such as chemical reactions,solubility, evaporation of metals,

catalytic processes, thermal denaturation of macromolecules, thermal killing of unicellular organisms and semiconduction processes.

Compensation effect has been widely investigated for different physical and chemical processes. Aguerre, Suarez, Viollaz (1986) [3] mentioned that early papers in this area was that of Bell (1937) who found a linear relation between the energy and entropy of solution for different solutes in the same solvent and of Everett (1950) who investigated this relation for the adsorption of vapors on charcoal and concluded that the entropy and heat of adsorption were linearly related. Other workers in this area as mentioned by Aguerre, Suarez, Viollaz (1986)[3] are Schreiner and Kembal (1953) and Cremer (1955). Schreiner and Kembal observed the parallelism between heat and entropy curves from measurements of adsorption isotherms of a number of vapors on oxides. However, the relation between the magnitudes was not determined by these authors. Cremer's study was related to heterogenous catalysis. This author introduced the term enthalpy-entropy compensation to designate the relationship between two magnitudes.

In small solute systems there are two general methods for generating the pairs of $\Delta S - \Delta H$ quantities which provide points of the compensation plot. The first way is to vary the chemical structure of a parent compound to produce a homologous series of reactants for a particular process, the second way is to vary the solvent composition. For protein

systems, both of these alternatives have been found to be effective ways to obtain the ΔH - ΔS pairs but in addition, it has also been found possible to vary the ΔS - ΔH values by changing the hydrogen-ion activity of the solution.

From the thermodynamic point of view, let's consider how one can theoritically get compensation from an analysis of thermodynamic laws.

Any chemical reaction can be separated into two part processes as chemical and solvation part process. For example, in the dissociation of weak uni-uni acid (HA) ;

Total process : $(HA)sol + water \longrightarrow (H^+)sol + (A^-)sol$

Chemical part process : $(HA)sol \longrightarrow \{H^+A^-\}$ (a)

Solvation part process : $\{H^+A^-\} \xrightarrow{water} (H^+)sol + (A^-)sol$ (b)

The chemical part process includes all inductive and resonance rearrangements of electrons to produce an electrostatic ion pair. The solvation part process includes the separation of the ions and all associated solvation effects. What such a separation into part processes says that solvent must rearrange itself around the reactants and products of a reaction in order to minimize the free energy. Although ΔH and ΔS may change rapidly with temperature they must be related by the general thermodynamic expressions

$$\Delta H = \Delta H^{\circ} + \int_{T_o}^{T} \Delta C_p dT \qquad (27)$$

$$\Delta S = \Delta S^{\circ} + \int_{T_o}^{T} (\Delta C_p/T) dT \qquad (28)$$

which give a strictly linear relationship between ΔS and ΔH only if $\Delta C_p = 0$. However $\Delta C_p \neq 0$ in the real world. Since the total thermodynamic expressions are;

$$\Delta G^{\circ} = \Delta G^{\circ}_a + \Delta G^{\circ}_b \qquad (29)$$

$$\Delta H^{\circ} = \Delta H^{\circ}_a + \Delta H^{\circ}_b \qquad (30)$$

$$\Delta S^{\circ} = \Delta S^{\circ}_a + \Delta S^{\circ}_b \qquad (31)$$

the quantities ΔH°_a and ΔS°_a also may depend on the nature of acid anion, therefore a considerable interpretation is required to abstract ΔH°_b and ΔS°_b . Thus the existence of a linear relationship was introduced [4].

$$\Delta H^{\circ}_b = \alpha + T_c \Delta S^{\circ}_b \qquad (32)$$

where intercept, α, is usually taken to be zero. T_c is not dependent on the experimental temperatures, it characterizes the compensation part process, however Lumry and Rajender (1970)[4] stated that intercept gives information about the chemical part process. According to Equation 32, one can say that in any reaction the solvent compensates changes in ΔH and ΔS for changes that to take place in the chemical part. Thus ΔG° varies little for a set of similar reactions of a homologous series or for the same reaction in a solvent changing pH or dielectric strength.

The compensation phenomena can be observed in a series of reactions by using $\Delta H^{\neq}$ and $\Delta S^{\neq}$ also, by plotting the measured values of $\Delta H^{\neq}$ from Arrhenius plots against the calculated values of $\Delta S^{\neq}$ from the transition rate theory equation, because both are proportional to the logarithms of rate constant [5].

A different argument was inverted by Kemeny and Rosenberg (1973)[5]. For reaction which yields very high activation entropies from the slope of Arrhenius plots, another positive exponential term should be added to the Arrhenius equation such as $\exp(\Delta S^{\neq}/R)$ to offset the extremely low pre-exponential values, $\exp(-\Delta H^{\neq}/RT)$, in order for such reaction rate constants to be measurable. Therefore $\Delta S^{\neq}$ is proportional in some way to $\Delta H^{\neq}$.

$$\Delta S^{\neq} = a \Delta H^{\neq} + b$$

From this relation, they concluded that only physically detectable processess of high activation enthalpies are those where a positive exponential factor operates and if the activation enthalpies empirically determined from good Arrhenius plots vary over a sufficiently wide range, the compensation law must be valid.

Enthalpy-entropy compensation analysis can be applied to a variety of systems, such as organic chemistry, protein-enzyme-water reactions, microbial death and food deterioration [6].

The most important study on compensation in protein and enzyme solutions have been made by Lumry and Rajender

(1970)[4]. According to these studies enthalpy and entropy change in a variety of protein reactions suggests that liquid water plays a direct role in many protein processes and linear enthalpy-entropy relationship is used as a diagnostic test for the participation of water in protein processes. They also pointed out the physiological importance of compensation for equilibrium and rate processes of proteins which exhibits Vaslow-Doherty effect which suggests that the compensation in unfolding is due to changes in the solvent to accomodate the non-polar groups of the unfolded polypeptide. H-bonded water clusters (i.e. ice-like structures) form with a large ΔC_p change. Thus T_c is not spurious. In some cases further change of solvent leads to $\Delta H^{\neq}$ / $\Delta S^{\neq}$ points to move back down the compensation line.

Real compensation effect in microbial death which results from protein denaturation is also a property of water/protein changes.

Recently Aguerre and his friends [3] applied the enthalpy-entropy compensation to the prediction of the effect of temperature on food isotherms obtained by other researchers and they showed that the data satisfied the $\Delta H/\Delta S$ compensation with the isokinetic temperature of 380.5 K.

An enthalpy-entropy compensation effect was also shown in the oxidation reduction reactions of bacterial,microsomal, and mitochondrial cytochromes P-450 [7].

In order to apply $\Delta H/\Delta S$ to food systems one needs data at some constant pH or water activity as a function of

temperature and data at some constant temperature as a function of water activity or pH. Labuza (1980)[6] says that the only ΔH / ΔS analysis of food chemical degradation have been represented by Kirk (1978) and Kirk and Dennison (1978). These authors studied the ascorbic acid degradation as a function of temperature and water activity. They observed the increase in activation energy with temperature and found T_c =274 K with r^2 = 0.999. Therefore the reaction was ΔS controlled which means larger changes in the solvent structure in the solvation part process are possible.

I.3. Aim of the Study

Enthalpy-entropy compensation is one of the most questioned occurrence in chemical reactions. As was briefly discussed in Section I.2, many of the studies in this area are performed in organic chemistry, in protein chemistry and in microbial death. Application of the enthalpy-entropy compensation to food deterioration reactions are very rare. Labuza (1980)[6] has tried to combine the works of several authors to examine the compensation effect, but there were no complete set of data since they were not reported for this purpose. It is clear that there is a great need for the analysis of compensation effect in food deterioration studies.

The aim of the present study was to examine the enthalpy-entropy compensation during ascorbic acid degradation in orange juice at different initial pH values.

For the purpose of comparison, same analysis were also planned for the degradation of pure ascorbic acid in aqueous media.

The method for this study may be outlined as following:

- At a constant temperature with different initial pH values, the concentration of ascorbic acid in orange juice and also in aqueous solution will be measured with respect to time .
- This procedure will be repeated at different temperatures.
- The rate constants will be obtained from the concentration-time data.
- Activation energies will be calculated from the Arrhenius plots at different pH values.
- Enthalpies of activation will be calculated from the activation energies and entropies of activation will be obtained from the frequency factors.
- Enthalpy-entropy plots will be prepared and isokinetic temperatures will be calculated from the slope for the degradation of ascorbic acid both in orange juice and aqueous solution.

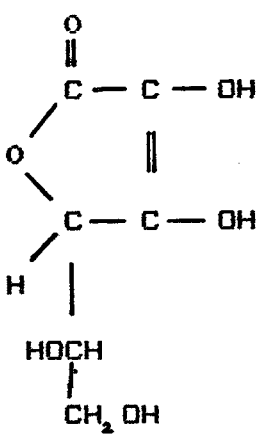
Besides showing the compensation between enthalpy of activation and entropy of activation, $\Delta H^{\ddagger}-\Delta S^{\ddagger}$ plots are also useful to predict the activation energies, frequency factors and thus the rates of reactions under different conditions. Since the main purpose of this study was to construct enthalpy-entropy plot for ascorbic acid degradation at various initial pH values, if isokinetic temperature, T_c ,

could be obtained from the slope of the line, then it would be very easy to get knowledge about the rates of ascorbic acid degradation at other pH values. A correctly prepared enthalpy-entropy plot, then, could be directly used to get this information instead of long experimentation.

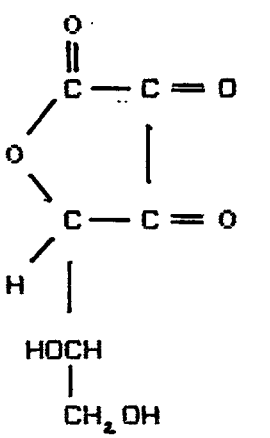
I.4. Information About Ascorbic Acid

Vitamin C occurs in nature as L-ascorbic acid, which is the generic name for L-threo-2-hexenono-1,4-lactone, in the form of white or slightly yellow crystals or powder. It is soluble in water, alcohol, glycerol and propylene glycol, but insoluble in chloroform, ether, benzene, xylene. At ordinary temperatures, dry crystals of ascorbic acid are stable on exposure to air.

Ascorbic acid has both acidic and strong reducing properties. These quantities are attributable to its enediol structure which is conjugated with the carbonyl group in a lactone ring.



L-ascorbic acid



L-dehydroascorbic acid

In solution, the hydroxyl on C-3 readily ionizes (pK =4.04 at 25°C) and a solution of the free acid gives a pH of 2.5. The second hydroxyl is much more resistant to ionization (pK = 11.4). The compound also absorbs moderately in the UV range and absorption is dependent on pH as shown in Table 1.

Table 1. UV absorption characteristics of ascorbic acid

pH	λ_{max}(nm)
2	244
6-10	266
> 10	294

Good results were obtained for its estimation in citrus juices with detection at 254 nm. At this wavelenght ascorbic acid has high absorbance and interference from coincidentally emerging natural constituents of the juice was insignificant. (Carnevale (1980))[81.

Vitamin C is widely distributed in nature, being found in many fruits especially the citrus, in green leafy vegetables and potatoes. Table 2 shows the typical concentrations of vitamin C found in a selection of fruit and vegetables.

Vitamin C has antiscorbutic activity and is essential to

man, who is unable to synthesize or store any significant quantity within the body and it is necessary that the diet contains a regular, adequate supply of this vitamin. Recommended daily allowance of vitamin C is 30 mg for an adult which is the recommended daily intake of vitamin C in various countries such as UK, Australia, Canada, Norway. Dietary levels for children varies from 15 to 30 mg according to ages respectively.

Table 2. Concentrations of ascorbic acid found in fruits and vegetables

	Ascorbic acid (mg/100 gr)
Acerola (West Indian Cherry)	1000-3000
Rose-hip	10-4800
Blackcurrant	90- 360
Porsley	154
Cabbage (raw)	70
Orange	30- 65
Lemon	39- 62
Grapefruit	37- 50
Tomato	10- 40
Apple	1- 35
Potato	2- 16

Since ascorbic acid is soluble in water, it is readily lost via leaching from cut or bruised surfaces of foods. However in processed foods, the most significant losses after processing result from chemical degradation. In foods which are particularly high in ascorbic acid, such as fruit products,loss is usually associated with nonenzymic browning. However in any processed foods, high concentration of ascorbic acid is due to use of it as a processing aid. Ascorbic acid can be used commercially in many of food products for two purposes. It can be added to enrich the products vitamin C content or it can be added to achieve some improvement in,for example,quality, shelf-life or processing, without regard for its vitamin activity in the end product. The main fields of applications are such that; it can be used instead of nitrites and nitrates during meat curing as an unquestionably safe nitrosamine inhibitor, in order to accelerate cured-meat pigment synthesis and to inhibit Clostridium botulinum growth. In addition it can be used as an improver in bread-making and since ascorbic acid functions as an oxygen scavenger, it offers significant functionality for hermetically sealed products containing air such as canned fruits and vegetables. In soft drinks especially beverages based on citrus fruits vitamin C functions as an antioxidant for the flavors as well as a nutrient. One another application is the use in beer industry as a stabilizer and it is effective especially when it is used together with sulfite [9,10].

There are several factors which influence the nature of the degradative mechanism such as temperature, salt and sugar concentration, pH, oxygen, enzymes, metal catalysts, amino acids, oxidants or reductants, initial concentration of ascorbic acid and the ratio of ascorbic acid to dehydroascorbic acid. Fig.2 shows the decomposition of vitamin C [11,13,9].

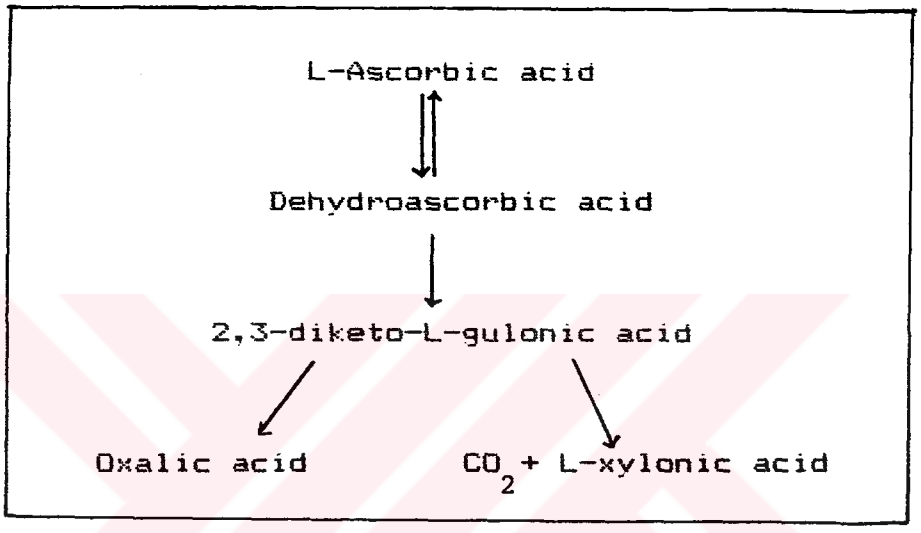


Fig.2 The decomposition of vitamin C

The first step of this reaction is reversible forming a redox system. One of the principal biochemical reactions of ascorbic acid is to destroy toxic free radicals, resulting from the metabolic products of oxygen. The mixture of L-ascorbic acid and its oxidation product, dehydroascorbic acid is thought of as a redox buffer. When terminating free radicals, ascorbic acid is converted to dehydroascorbic acid, which is then recycled to ascorbic acid by reductase enzymes and cofactors.

Ascorbic acid is oxidized either by one or two-electron

transfer [12, 13].

One-electron reduction :

When one electron is transferred in the rate limiting step, the first product formed from L-ascorbic acid is the L-ascorbic acid radical ($AH^{\cdot}$). It is a strong acid with pH 0.45 and at a physiological pH it exists as a radical ion ($A^{\cdot-}$).

Free radicals that damage lipids and other readily oxidized compounds in foods and tissue are terminated by reduction with L-ascorbate. In the reaction, one electron is transfered to the free radical from ascorbate giving ascorbate radical. Ascorbate radical is the initial oxidation product of two enzyme reactions that occur in plants. Ascorbate oxidase is the enzyme that oxidizes L-ascorbic acid [14] and in the presence of oxygen, the overal reaction is;

$$2\ \text{Ascorbate} + O_2 \longrightarrow 2\ \text{Dehydroascorbic acid} + 2H_2O$$

Optimum pH range for ascorbate oxidase is 5.5-7.0.

Ascorbate peroxidase is another plant enzyme that oxidizes ascorbic acid, but it uses H_2O_2 instead of O_2 as the electron acceptor.

$$\text{Ascorbate} + H_2O_2 \longrightarrow \text{Dehydroascorbic acid} + 2H_2O$$

Two-electron reduction :

The reaction of L-ascorbate with dioxygen in the presence of transition metal ions is of prime importance. The transition metals, especially cupric and ferric ions,

catalyze the autoxidation by joining together L-ascorbate and oxygen in a proposed ternary complex.Then, two π-electrons from L-ascorbate in the complex are thought to shift to oxygen through the transition metal ion. The complex dissociates, and the products are dehydroascorbic acid, H_2O_2 and metal ion. In most foods dehydroascorbic acid is rapidly hydrolyzed to 2,3 diketogulonic acid by a non-reversible reaction and results in loss of vitamin C activity [12].

In the alkaline region, oxidation is directly proportional to partial pressure of oxygen. However, at pH:7 and below, the reaction rate was proportional to a power of oxygen partial pressure of 0.4 [15, 13].

The uncatalyzed reactions is not proportional to O_2 concentrations at low partial pressures of O_2, below 0.4, the rate seems to level off. In contrast, the rate in the catalyzed pathway is proportional to O_2 concentration for partial pressures down to 0.19 atm. In both catalyzed and uncatalyzed reactions O_2 takes place in the rate determining step.

The pH-rate profile for uncatalyzed oxidative degradation is a S-shaped curve which increases continuously through the pH corresponding to pK of ascorbic acid [16] and then tends to flatten out above pH:6. This is taken as evidence at pH:7 and below, primarily the monoanion participates in oxidation. Related to this, Perchonok and Downess (1982) suggested that, in alkaline pH only the divalent ion of ascorbic acid participated in the oxygen

absorption [15]. These authors and Singh (1976) [17, 15] discussed also that when dissolved oxygen was maintained at saturation, the reaction followed a first order mechanism. However, with limited oxygen, the reaction followed second order kinetics.

Under anaerobic conditions the rate reaches to maximum at pH 4, declines to a minimum at pH:2, and then increases again with increasing acidity. Although the anaerobic pathway can also contribute to ascorbic acid degradation in the presence of O_2, even the uncatalyzed oxidative rate is very much greater than the anaerobic rate at ambient temperatures. Therefore, both pathways may be operative in the presence of O_2 with the oxidative pathways being dominant. In the absence of O_2 there is no added influence of metal catalysts. However, certain chelates of Cu^{+2} and Fe^{+3} are catalytic in a manner independent of O_2 concentration, with catalytic effectiveness being function of metal chelate stability.

In foods such as canned juices, the loss of ascorbic acid tends to follow the first order reaction until the dissolved or headspace O_2 is completely exhausted then it may follow anaerobic degradation. In dehydrated citrus juices, degradation is the function of only temperature, and moisture content. Although ascorbic acid appears to be degraded even at very law moisture contents, the rate becomes slow that the long storage with an acceptable loss can be used.

Although stability of ascorbic acid increases with lower temperatures, storage temperature above -18°C leads to

signifiant losses on frozen storage. For noncitrus foods, the largest losses will occur during heating. However the leaching loss during heating exceeds the losses during other process steps.

I.5. Methods for Determination of Ascorbic Acid

There are several methods for the determination of vitamin C [18] such as titration methods using suitable oxidizing agents like 2,6-dichlorophenolindophenol, iodine, chloramine-T, N-bromosuccinimide, sodium 1,2-naphthoquinone-4-sulphonate,hexamminecobalt(III)tricarbonatocobaltate(III), potasium ferricyanide,complexometric method,microfluorometric assays, spectrophotometric determinations, determination with diazotized 4-methoxy-2-nitroaniline,diazotized p-nitroaniline or potasium ferricyanide, photometric determination with 4-nitrobenzene diazonium fluoroborate, colorimetric determinations using 3,4-dinitrobenzoic acid, 2,3,5-triphenyltetrazolium chloride, phosphomolybdate complex, photometric titration with potassium ferricyanide, polarographic determinations in buffer solutions of different pH values, potentiometric determinations with iodine or copper sulphate, assays after isolation by paper chromatography, determinations by thin-layer chromatography, determinations by gas chromatography and determinations with HPLC.

HPLC is the most commonly preferred method and it can be used for determination of ascorbic acid, dehydroascorbic acid

or determination of both for estimation of total ascorbic acid in food materials.

Analysis for the content of ascorbic acid in food products involves the extraction of ascorbic acid and measurement by HPLC. Several extraction methods have been reported using metaphosphoric acid, EDTA, citric acid, citric acid and EDTA [19]. However, direct water extraction was used especially for juices [19,20,21,22]. Direct extraction from juices mainly involves the steps juicing, centrifuging, filtering, diluting and injecting to HPLC.

The columns and the mobile phase used vary according to the food material analyzed, purpose of analysis and method of analysis.Mainly the colums used are μ-Bondapak NH_2, μ-Bondapak C-18 [23,24,25],Zorbax NH_2[26], Spherisorb ODS [24], Partisil 10SAX, μ-Bondapak CN [18]. μ-Bondapak CN was used by Carnevale (1980) in determination of sorbic, benzoic and ascorbic acid in citrus juices with the mobile phase of 2% acetic acid/methanol (19:1) [24].

Rose, Nahrwold (1981) used Lichrosorb-NH column with the mobile phase consisted of 2.5 mM KH_2PO_4/CH_3 CN(50/50) in quantitative analysis of ascorbic acid and dehydroascorbic acid by HPLC equipped with a UV absorbance detector [20].

Dennison and his friends (1981) used μ-Bondapak-NH_2 column with the mobile phase 50:50 (v/v) methanol/0.25 % KH_2PO_4 buffer for determination of ascorbic acid and combined ascorbic acid - dehydroascorbic acid determination in beverages [21].

A Brownlee RP-18 guard column and Altex Ultrasphere ODS (C 18) were used in determination of ascorbic acid, erythorbic acid and uric acid in cured meats by Kutnink and Omaye (1987) [27].

Wimalasiri and Wills (1983) used μ-Bondapak/Carbohydrate column in simultaneous analysis of ascorbic acid and dehydro-ascorbic acid in fruit and vegetables with the mobile phase of acetonitrile-water (70:30, v/v) with 0.01 M ammonium dihydrogen phosphate [28].

Russell (1986) applied the HPLC method for analysis of vitamin C in fresh tomatoes using Vydac 201 HS column and 0.5 mM tridecylammonium formate in 60+40+1 methanol + water + acetonitrile (pH:4.25) as mobile phase [29].

CHAPTER II

EXPERIMENTAL

II.1. Preparation of Orange Juice Samples

Since the concentration of ascorbic acid in each orange varies according to season, harvesting, maturity, cultivar, climate, duration of storage, thus, there may be differences in the results of two subsequent experiments due to properties of the analyzed oranges. In order for eliminating this disadvantage, two boxes of oranges were bought and each fruit was wrapped with aluminum foil to prevent moisture loss and then stored in the refrigerator approximately at 4°C until experimental usage.

In sample preparation each fruit was squeezed manually and centrifuged in model Hettich Roto Silenta III for 5 min, in order to precipitate the excessive pulp. The supernatant was poured into a beaker and pH was measured with model Metrohm Herisau Digital pH-meter E 500. Original pH was found between 3-3.5. Then, it was adjusted to one at which experiments would be performed with a few drops of concentrated NaOH for alkaline pHs compared to the measured

pH of sample or with concentrated citric acid for acidic pHs. The sample prepared was filled into a brown bottle and put into a thermostat adjusted to the specified temperature. The time passed during sample preparation was 10-15 min.

The thermostat (generally referred to as water bath in this text) was constructed specially for this study. It was mainly a cylindrical copper container (radius: 25 cm, height: 26.5 cm) , which was jacketed with another cylindrical copper container (radius: 27.5 cm, height: 31 cm). The space between the two containers was filled with glass wool for insulation. Temperature control was achieved by a Heidolph model temperature controller. The thermostat was covered with a thick foam plate to prevent the heat losses. In this way, the temperature was controlled to ± 0.1°C.

The water bath was placed into a dark room in order to prevent the light effect on ascorbic acid loss. After placed into water bath, the temperature of the sample was allowed to reach to the temperature of water bath. That time was noted as the starting time. After this time 10-15 ml samples were removed from the reaction bottle in 10-20 min. intervals for analysis.

II.2. Preparation of Aqueous Ascorbic Acid Samples

For the preparation of aqueous ascorbic acid solutions at different pH values, 50 mg of solid ascorbic acid (Sigma Chemical Company, No: A-278) were added to each of four 100 ml citrate buffer solutions to obtain final pH values of

about 2.38, 3.69, 4.90, 5.82. The solution prepared was filled into a brown bottle and placed into water bath. 10-15 ml of solution was taken in 10-20 min. intervals after the thermal equilibrium was reached and analysis were performed.

II.3. Experimental Procedure for the Determination of Ascorbic Acid

In this work, determination of ascorbic acid was made by the use of HPLC which provides the most sensitive and rapid determination. Just like all chromatographic separations, HPLC separations are based upon differences in the extent to which solutes are partitioned between the mobile and stationary phases.

In HPLC method the sample injected is carried by the moving stream of liquid known as mobile phase through the stationary phase which is the column packed with porous paper or finely ground solid or is an immobilized liquid immiscible with mobile phase under the pressure.

The components to be separated must be soluble in the mobile phase. They must also be capable of interacting with the stationary phase either by dissolving in it, being adsorbed by it, or reacting with it chemically. Therefore the separations are based on the differences in migration rates among the sample components. In another words, separation depends on the retention time of each component which is the time required for the sample injected to pass through the column.

The vast increase in the number of analytical methods involving HPLC is due to ;

1) rapid determination with high selectivity and accuracy,
2) requirements of minimum sample preparation,
3) direct analysis without derivatization,
4) simultaneous determination of several components in a single analysis and the resolution of isomers,
5) shortening time of analysis.

The HPLC used in the analysis was a Water Associates liquid chromatography, equipped with Model 6000A pump, a Model U6K injector, and a Model 440 UV absorbance detector.

Model 6000A is a high performance solvent delivery system having maximum output pressure of 6000 pounds per square inch gauge (psig) with the overpressure limit adjustable from 100 to 6000 psig.

Constant flow of the solvent delivery is achieved with a pair of specially-driven positive displacement pumping chambers.Electronic control of piston reciprocation rate governs the solvent flow rate. Flow rate is manually selectable and it was maintained at 1.3 ml/min throughout the experiments.

Because of the low internal volume of Model 6000A, solvent change-over time is minimized and recycle operation is permitted. For example, each piston in the pair of pumping chambers only displaces 100 microliters of solvent per stroke.

The U6K injector enables the user to load samples and make injections at system pressure up to 6000 psi. It can accomodate the full range of injections, from a fraction of a microliter to 2 ml.

Although sample is loaded at atmospheric pressure, the solvent is simultaneously being delivered to the system at full pressure. Before injection, solvent flow path is changed by turning the sample handle to the injection position. This also opens the sample loading loop to the atmosphere making it possible to displace solvent,which is already in the loop, with sample as it is delivered from the syringe. After injection is performed by means of a syringe, this time the sample handle is turned to loading position and solvent flow is directed through the sample loading loop to the column carrying the sample. The column effluents are monitored at 254 nm, with a Model 440 dual beam UV-visible absorbance detector. The conditions for HPLC are summarized in Table 3.

A Novapak C_{18} packed column with column size of 3.9 mm x 15 cm was used and sharp peaks were obtained for ascorbic acid. The mobile phase used is also given in Tables 3. It is contained in approximately 1 lt glass reservoirs with which the HPLC apparatus was equipped. The reservoir was equipped with a degasser which was a vacuum pumping system for removing dissolved gases that interfere by forming bubbles in the column and the detector systems. These bubbles cause band spreading. In addition, they often interfere with the performance of the detector.

Table 3. HPLC working conditions for the determination of ascorbic acid

Column	: Novapak C_{18} (3.9 mm x 15 cm)
Mobile phase	: Methanol/water (20:80),pH=5-5.5
Flowrate	: 1.3 ml/min
Wavelength of measurement	: 254 nm
Retention time (cm)	: 0.6
Sensitivity	: 0.1 AUFS
Chart speed	: 10/10 cm/min

During sample injection a 10-15 ml of sample was taken from the reaction bottle placed into the water bath by means of a pipette and cooled under tap water to room temperature.

It was then immidiately passed through the sample clarification kit which was mainly a 10 ml glass syringe including a filter holder system. Both a glass fibre prefilter and a cellulose acetate filter (Millipore Corp.) were placed into the filter holder system and the sample was filtered before injection. Direct dilution and injection of sample was prefered and applied by several authors for orange juice [20, 22].

Therefore the clear supernatant obtained in this way was diluted with double distilled water in a ratio of 1/60 to 1/100 depending on the concentration of ascorbic acid in each

sample. Then by using a glass syringe, 10 µl of the sample was directly injected to HPLC more than once to be able to get avarage peak height.

All this procedure from water bath to injection was performed within 7-9 min. Concentration of ascorbic acid in the sample was calculated from the peak height of the ascorbic acid in the chromatogram. A calibration curve of peak height versus concentrations of standard ascorbic acid solution was used for the conversion of the peak height of ascorbic acid in the sample to its concentration.

CHAPTER III

RESULTS

III.1. Preparation of the Calibration Curve for Ascorbic Acid

At the begining of the study, a calibration curve was prepared by dissolving suitable amounts of ascorbic acid in double distilled water and injecting known amounts into HPLC in order to determine the peak heights. But the peak heights obtained from the injection of the same amounts of ascorbic acid were not reproducible and they were showing decreasing trends. This effect was also seen in the calibration curve so that there was a deviation from the linearity towards the higher concentrations of ascorbic acid. The reason for this deviation was mainly the oxidation of L-ascorbic acid to dehydroascorbic acid.

Literature survey indicated that dithiothreitol [26] and homocystein [20] could be used as agents to prevent the conversion of L-ascorbic acid to dehydroascorbic acid.

Therefore, dithiothreitol, which was already present in our laboratory was used in the preparation of standard ascorbic acid solution

For the preparation of standard ascorbic acid stock solution, 100mg of DL-dithiothreitol (Sigma Chemical Company No.D-0632) was dissolved in 100 ml of distilled water in 1:1 (mg/ml) ratio and about 50 mg of pure solid L-ascorbic acid was added. Dithiothreitol addition completely stabilized the L-ascorbic acid and reproducible results were obtained for at least two hours.

Then by succesive dilutions, various standard solutions of ascorbic acid were obtained 10 µl from each of solutions was injected directly into HPLC for the determination of peak height. Retention time, in cm., of ascorbic acid was 0.6 as given in Table 3.

Actually the calibration curve for ascorbic acid was checked frequently during the analysis by preparing new standard solution and by measuring the peak heights at different concentrations. There was an excellent agreement between the previous line and the new points for ascorbic acid. Figure 3 shows the calibration curve used throughout the experiments.

III.2. Collected Data from the Analysis of Ascorbic Acid Degradation in Orange Juice Samples

The change in the concentration of ascorbic acid in orange juice was followed at temperatures 290.5, 295, 303, 308 and 313 K. At each temperature, the pH of the original orange juice was adjusted to about 2,3,4 and 5. After heating at a certain temperature and pH value for various reaction

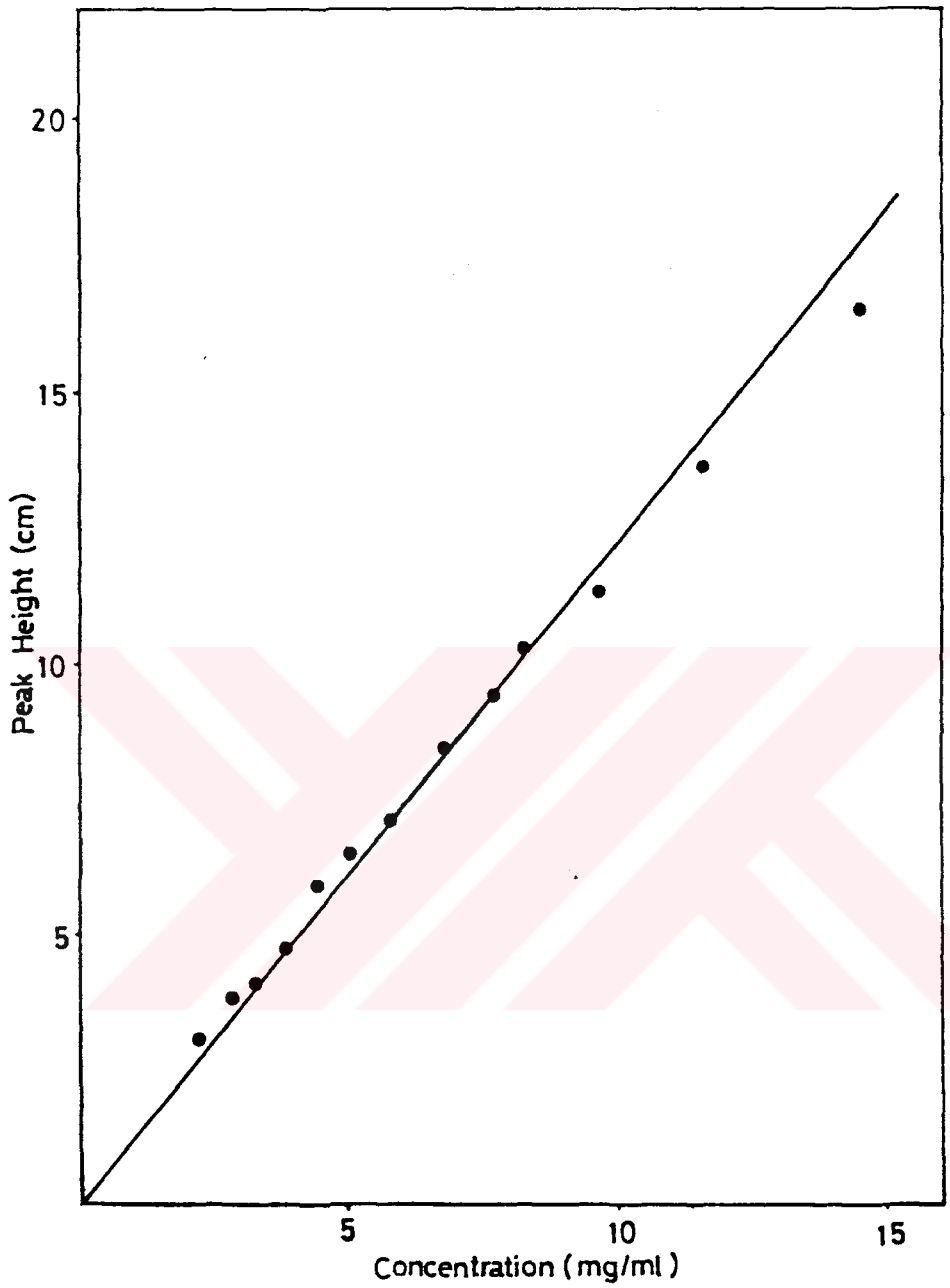


Figure 3. Calibration curve for ascorbic acid used throughout the experiments.

times, the sample was treated as explained in Section II.3 and the concentrations of ascorbic acid were calculated by using the calibration curve. Collected data of ascorbic acid concentration versus time in orange juice are given Table 4.

III.3. Collected Data from the Analysis of Ascorbic Acid Degradation in Aqueous Solution

The change in the concentrations of ascorbic acid in aqueous solution was examined at temperatures 303, 313, 323 and 333 K. At each temperature, the pH of the original aqueous ascorbic acid solution was adjusted to about 2.4-3.7, 4.9 and 5.9 by the procedure explained in Section II.2. After the suitable reaction times, samples were analyzed by HPLC and calibration curve was used to obtain the concentration of ascorbic acid. Table 5 includes the collected data of ascorbic acid concentration versus time in aqueous solution.

III.4. Treatment of Data and Results

After the collection of concentration-time data, the rate constants for the degradation of ascorbic acid in both the orange juice and in aqueous solution were calculated.

Integral method of analysis of data was used for this purpose and as suggested by many scientists [30,17,15,16,31] it was assumed that under the present experimental conditions, ascorbic acid loss followed a first-order kinetics. Then from the integrated rate expression for the first order reactions

Table 4. Collected data of ascorbic acid concentration versus time in orange juice

T(K)	pH	Time (min)	Concentration of ascorbic acid (mg/ml)	k(min^{-1})	r
313	2.05	14	0.616		
		24	0.592	0.00208	0.979
		50	0.560		
		69	0.548		
	3.05	13	0.320		
		27	0.308	0.00296	0.999
		44	0.292		
	4.04	27	0.540		
		39	0.516	0.00352	0.999
		54	0.492		
		72	0.460		
	5.05	18	0.984		
		30	0.940	0.00258	0.983
		43	0.912		
		54	0.896		
308	2.04	13	0.228		
		24	0.225	0.00153	0.999
		49	0.216		
		62	0.212		
	3.04	0	0.242		
		12	0.228	0.00235	0.900
		23	0.222		
		36	0.222		
	4.04	13	0.459		
		36	0.462	0.00266	0.860
		61	0.405		
	5.04	14	0.474		
		37	0.456	0.00200	0.996
		64	0.429		
303	2.04	13	0.280		
		27	0.276	0.00059	0.887
		39	0.276		
	3.04	12	0.552		
		26	0.560	0.00161	0.850
		39	0.532		
		50	0.524		

Table 4. (cont'd)

	4.04	14	0.336		
		39	0.328	0.00225	0.830
		45	0.308		
	5.04	0	0.454		
		11	0.448		
		24	0.436	0.00181	0.989
		38	0.420		
		55	0.416		
		69	0.400		
295	4.04	22	0.348		
		32	0.341	0.00216	0.932
		52	0.334		
		61	0.316		
290.5	3.04	18	0.430		
		28	0.421	0.00148	0.940
		34	0.416		
		42	0.416		
	5.05	0	0.620		
		8	0.610	0.00154	0.974
		16	0.597		
		34	0.588		

Tablo 5. Collected data of ascorbic acid concentration versus time in aqueous solution

T(K)	pH	Time (min)	Concentration of ascorbic acid (mg/ml)	k(min^{-1})	r
333	2.38	61	1.050		
		114	0.865		
		160	0.825	0.00156	0.915
		196	0.810		
		231	0.790		
	3.63	61	0.280		
		111	0.261		
		155	0.238	0.001787	0.979
		190	0.235		
		225	0.216		
		255	0.193		
	4.88	61	0.477		
		107	0.420		
		149	0.395	0.00198	0.985
		183	0.387		
		216	0.345		
		252	0.320		
333	5.88	54	1.170		
		99	1.070		
		141	0.995	0.00182	0.995
		175	0.955		
		206	0.875		
323	2.28	18	0.119		
		92	0.107	0.00106	0.973
		121	0.107		
		147	0.104		
	4.88	15	0.400		
		30	0.392	0.00164	0.999
		89	0.355		
		143	0.325		
	5.78	62	1.030		
		77	0.990	0.00144	0.964
		108	0.960		
513	2.41	96	0.980		
		142	0.950		
		181	0.900	0.00619	0.938
		220	0.905		

Table 5. (cont'd)

		253	0.890		
	3.68	52	0.353		
		93	0.311		
		137	0.295	0.000964	0.848
		176	0.288		
		249	0.287		
	4.91	45	0.560		
		89	0.530		
		131	0.513	0.00129	0.995
		173	0.472		
		208	0.453		
		211	0.438		
	5.90	38	1.005		
		84	0.955		
		127	0.945	0.00118	0.970
		165	0.870		
		198	0.875		
		230	0.825		
303	2.44	0	1.290		
		165	1.200	0.000438	0.970
		205	1.210		
		307	1.220		
	3.76	57	0.370		
		104	0.362	0.000613	0.967
		156	0.358		
		297	0.320		
	4.93	52	0.583		
		97	0.575	0.00076	0.985
		190	0.535		
		243	0.505		
	5.72	0	1.320		
		96	1.210	0.00093	0.998
		143	1.150		
		185	1.115		

$$-\ell n\ C_A = -\ell n\ C_{Ao} + kt$$

where C_A is the concentration of ascorbic acid, C_{Ao} is its initial concentration and k is the rate constant; graphs of $-\ell nC_A$ versus time were prepared at each constant pH and temperature condition.

III.4.1. Results of Ascorbic Acid Loss in Orange Juice

Figures from 4 to 18 represent the linear relation of $-\ell nC_A$ to time at different temperature and pH conditions.

The rate constants for the loss of ascorbic acid in orange juice were determined from the slopes of these plots by the method of linear least squares analysis.

Table 4 also summarizes the calculated rate constants at different temperature and pH values.

The rate constant given in Table 4 are in reasonable agreement with pH and temperature changes. The limited number of data at temperatures 290.5 and 295 resulted because of the elimination of some experimental observations which were found to be too much scattered.

These data of rate constants might also be examined by plotting the rate constants with respect to pH at each temperature. Figure 19 shows the change of rate constants of ascorbic acid loss in orange juice by the variations in pH at temperatures 290.5, 295, 303, 308 and 313 K.

As it is seen in Figure 19, the rate of degradation of

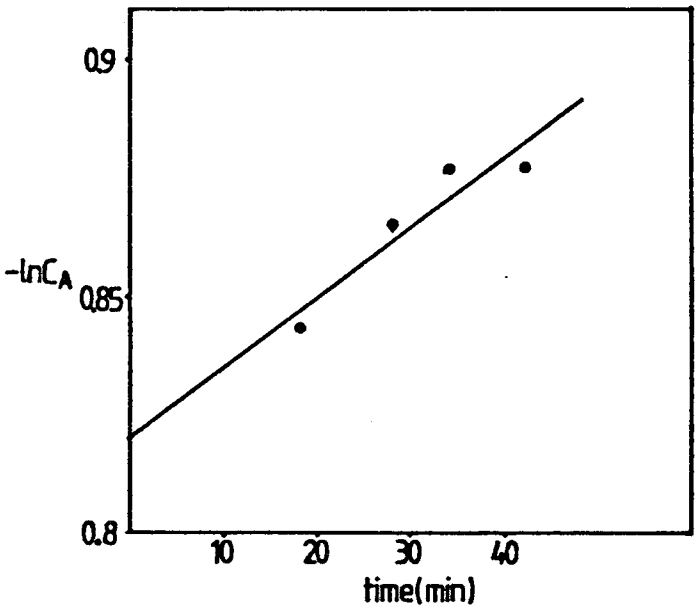


Figure 4. Ascorbic acid loss in orange juice at T =290.5 K,pH: 3.04.

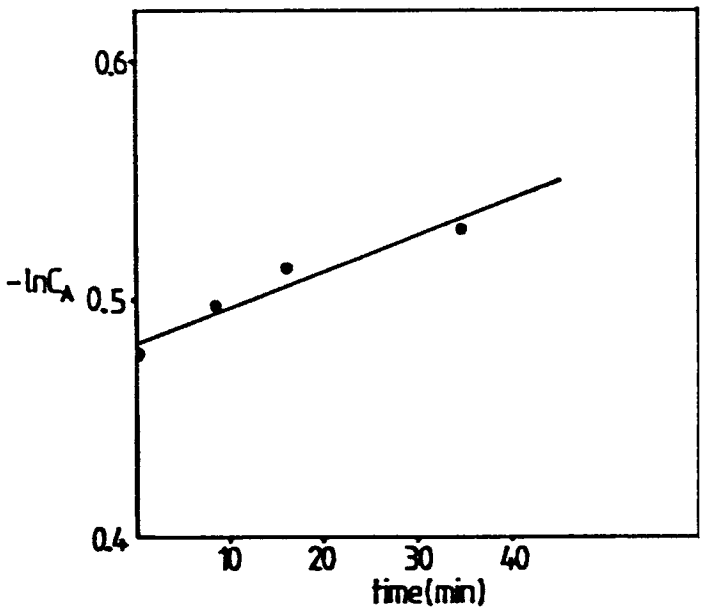


Figure 5. Ascorbic acid loss in orange juice at T =290.5 K,pH: 5.05.

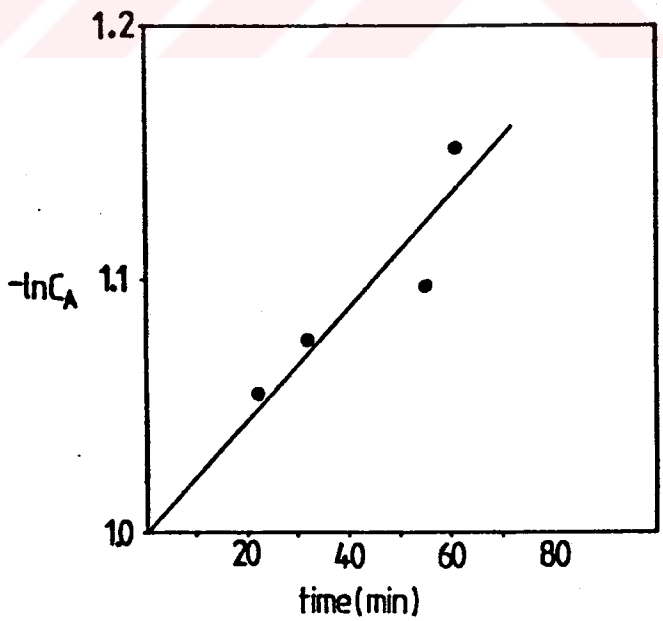


Figure 6. Ascorbic acid loss in orange juice at T=295K,pH : 4.04.

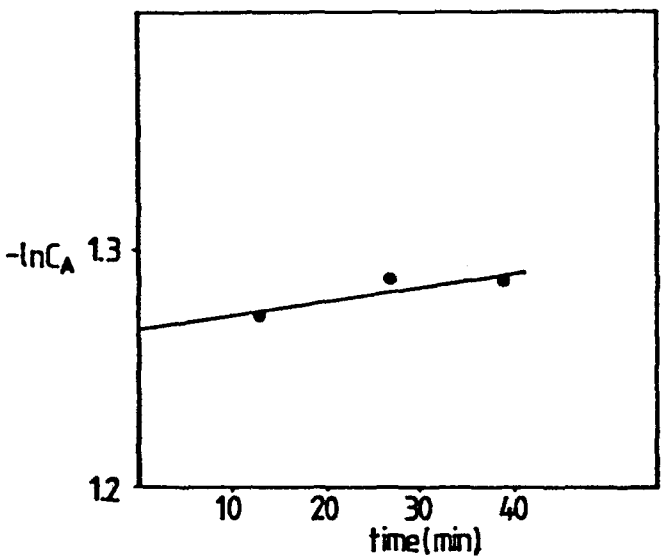


Figure 7. Ascorbic acid loss in orange juice at T=303 K,pH:2.04.

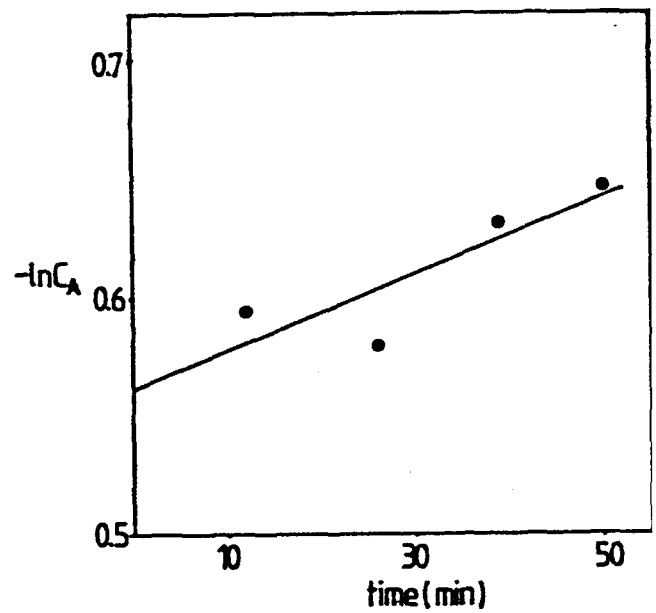


Figure 8. Ascorbic acid loss in orange juice at T=303 K,pH:3.04.

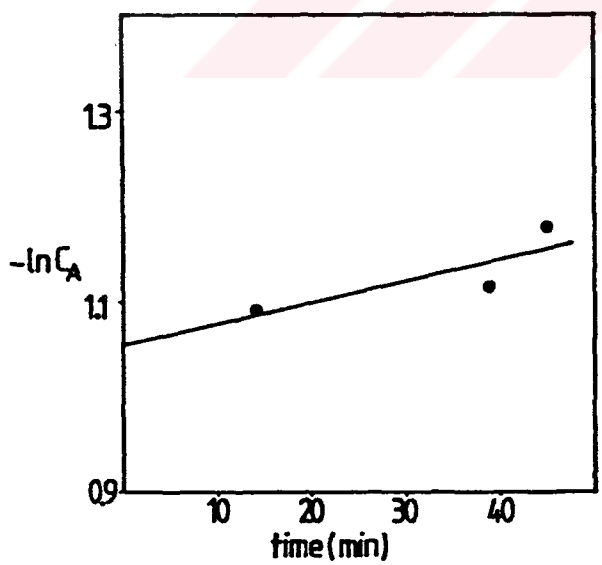


Figure 9. Ascorbic acid loss in orange juice at T= 303 K, pH:4.04.

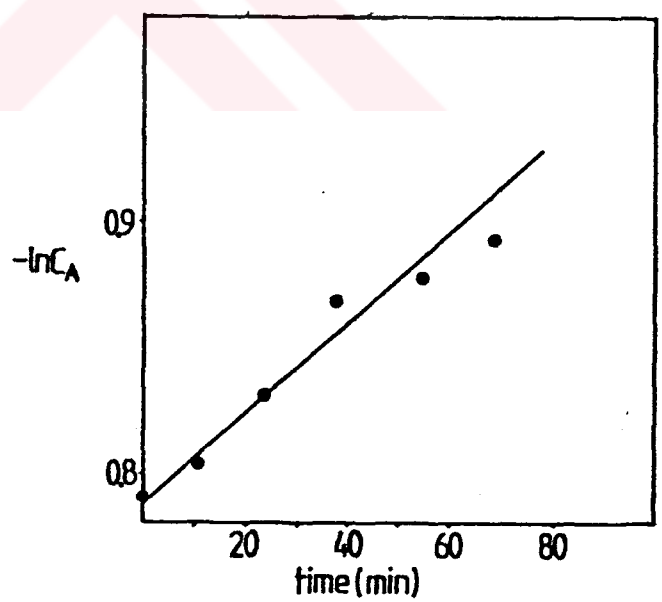


Figure 10. Ascorbic acid loss in orange juice at T=303 K, pH:5.04.

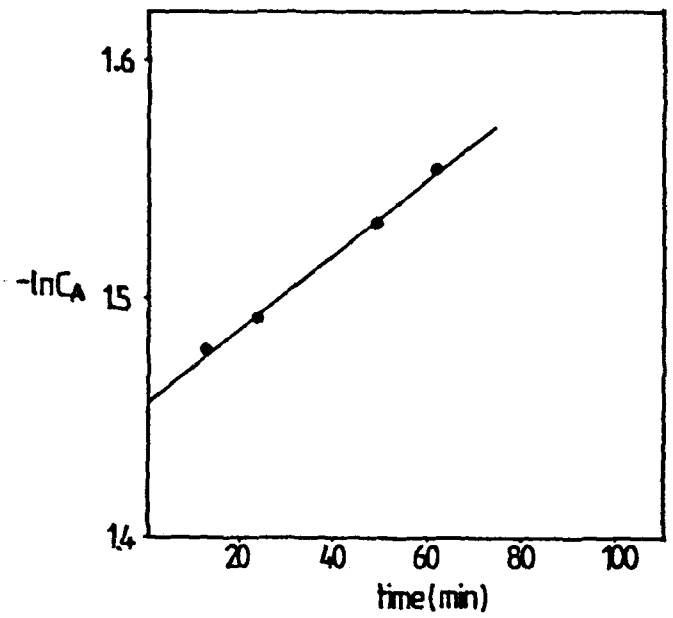


Figure 11. Ascorbic acid loss in orange juice at T= 308 K,pH:2.04.

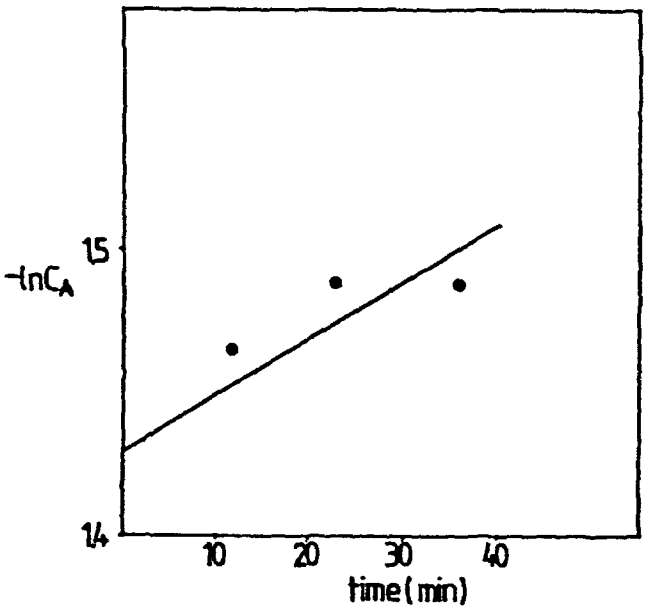


Figure 12. Ascorbic acid loss in orange juice at T= 308 K,pH:3.04.

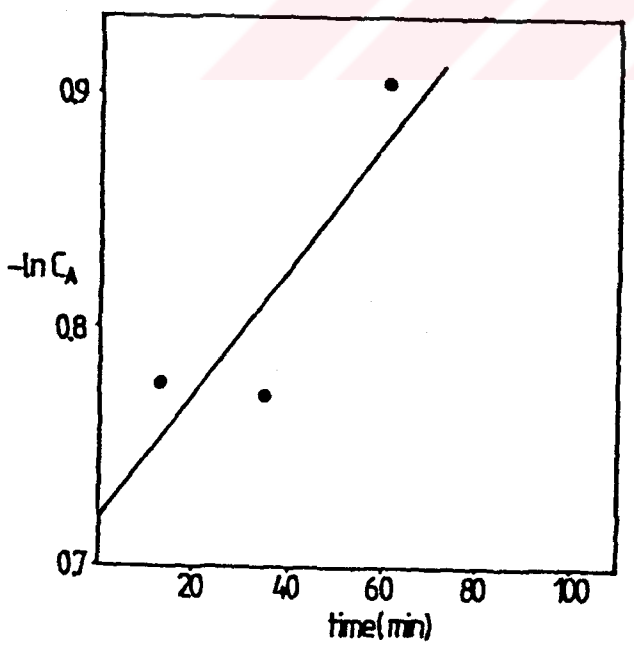


Figure 13. Ascorbic acid loss in orange juice at T=308 K,pH:4.04.

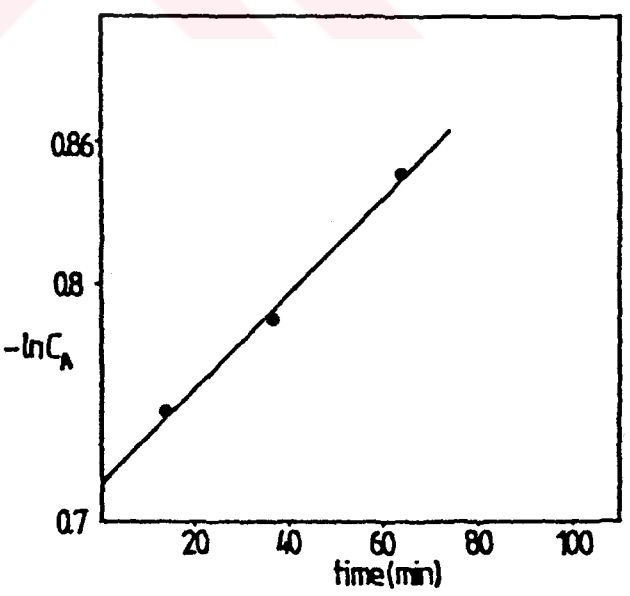


Figure 14. Ascorbic acid loss in orange juice at T=308 K,pH:5.04.

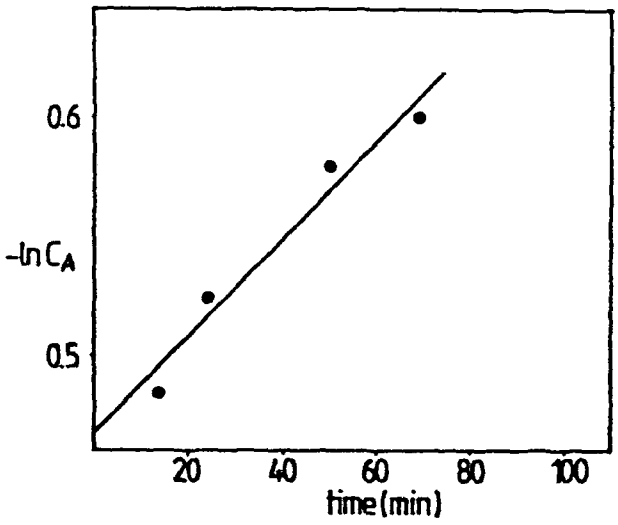


Figure 15. Ascorbic acid loss in orange juice at T=313 K,pH:2.05.

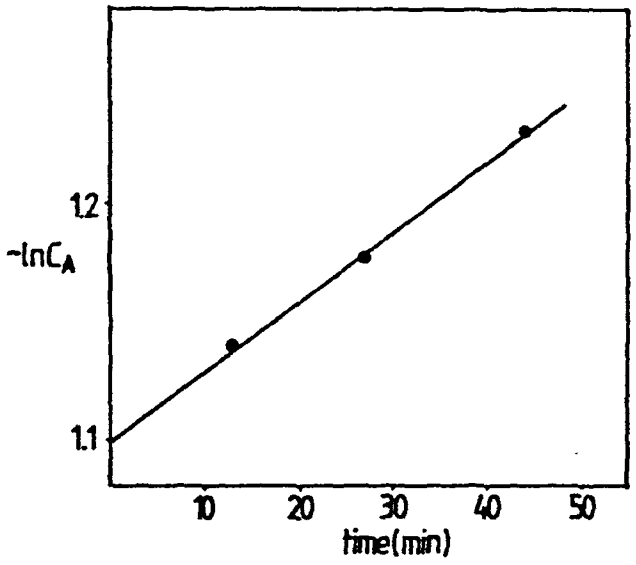


Figure 16. Ascorbic acid loss in orange juice at T= 313 K,pH:3.05.

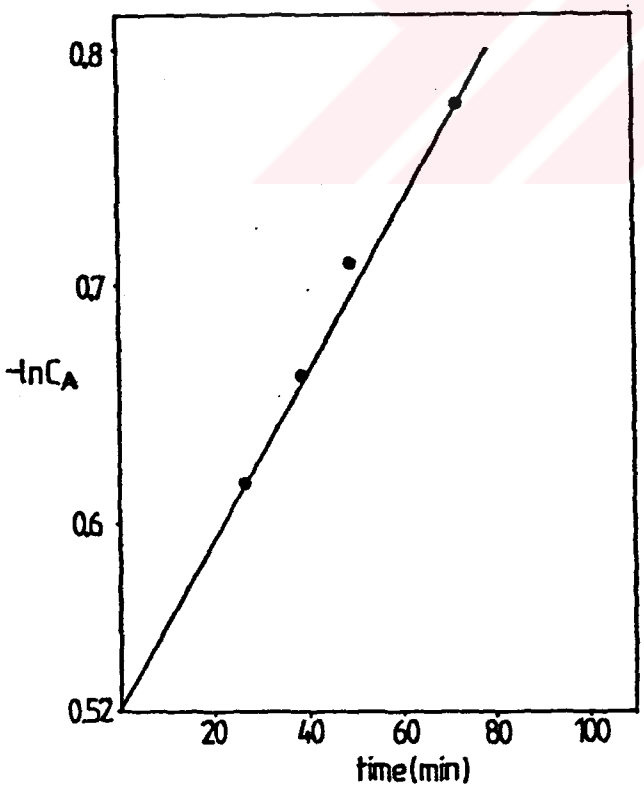


Figure 17. Ascorbic acid loss in orange juice at T=313 K,pH:4.04.

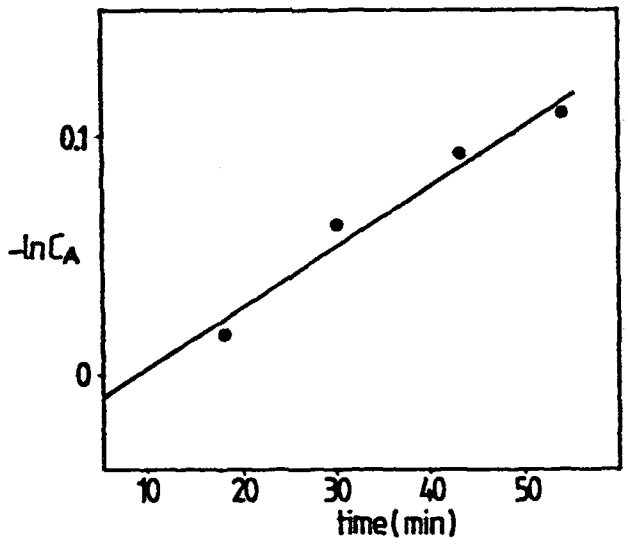


Figure 18. Ascorbic acid loss in orange juice at T=313 K,pH:5.05.

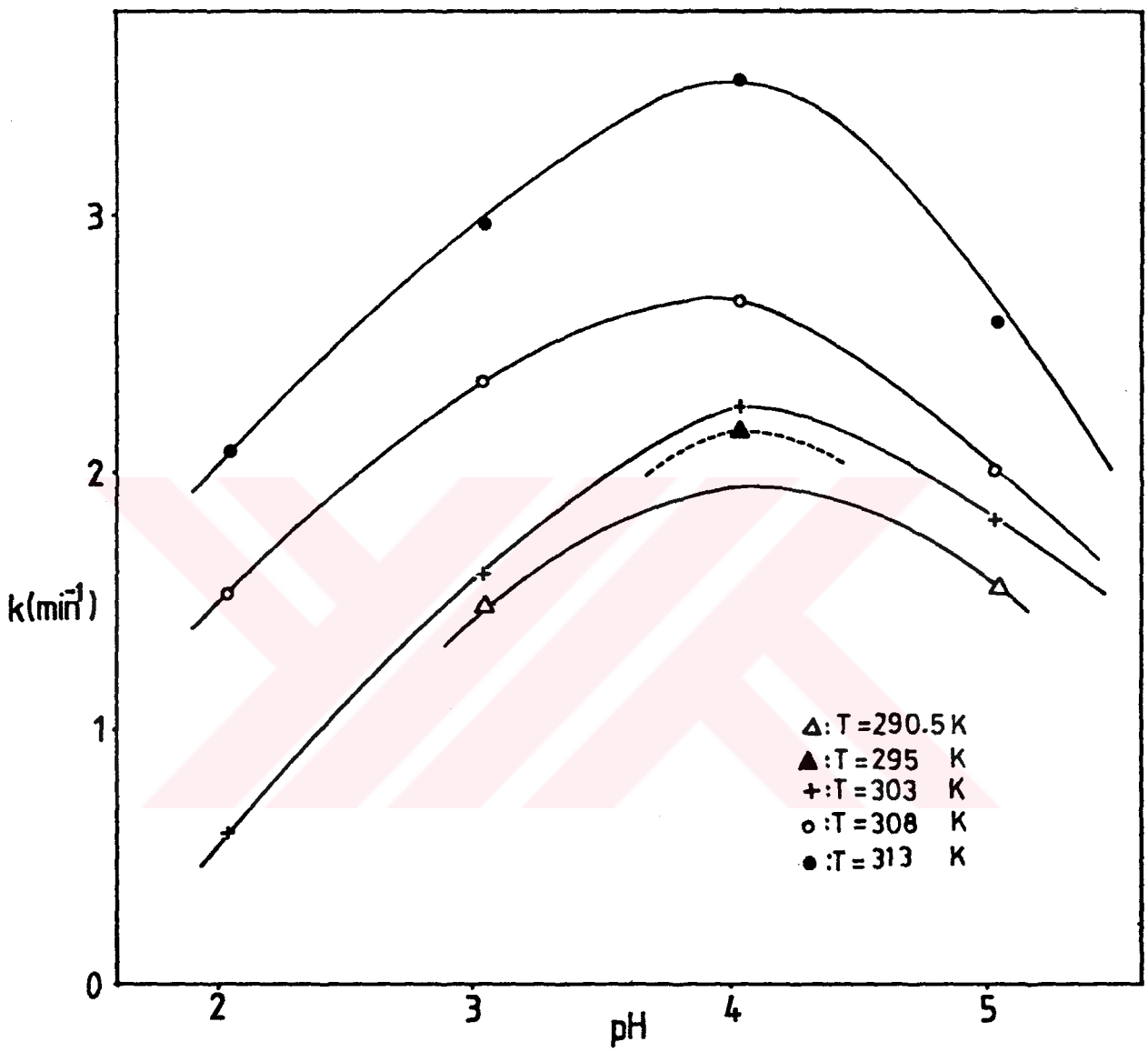


Figure 19. The plot of rate constant versus pH for ascorbic acid loss in orange juice at different temperatures.

ascorbic acid becomes larger as the pH of the initial orange juice is increased from 2 to 4.This trend is same at all four temperatures. However when the initial pH is further increased to 5, a decrease in the rate of loss is observed at each temperature. This plot (Figure 19) suggests that, when the pH of orange juice is 4, maximum loss of ascorbic acid occurs. Although there are only two rate constant data at temperature 290.5 K and one at 295 K same kind of curve might be expected in the plot. Figure 19 is mainly helpfull to see the effect of temperature on ascorbic acid loss at the same pH values.

Activation energies at each pH value should be obtained from the plots of $-\ell nk$ versus $1/T$ (K^{-1}). Figures 20-23 are the Arrhenius plots for ascorbic acid loss in orange juice at four different pH values.

Table 6 includes the activation energies, intercepts and correlation coefficients calculated from the Arrhenius plots by the method of linear least squares analysis.

The next step in the analysis of enthalpy-entropy compensation is to calculate the values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ from the kinetic parameters presented in Table 6. According to the derivations shown in Section I.1.,

$$E_{exp} = \Delta H^{\neq} + RT \qquad (26)$$

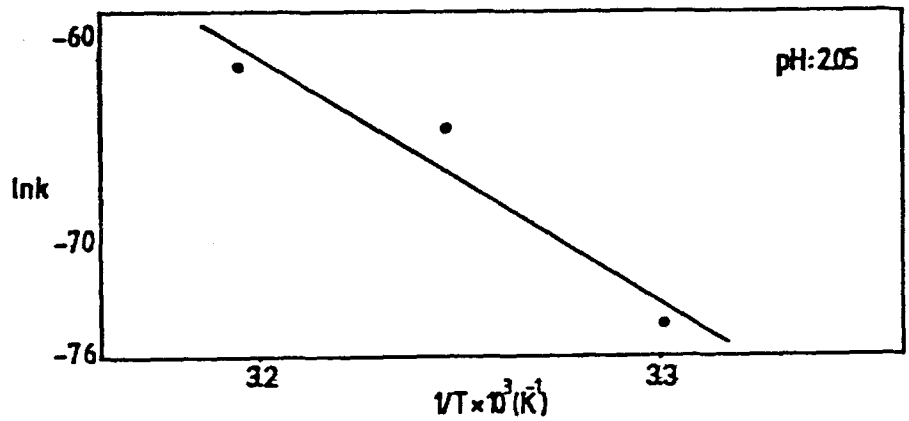


Figure 20.Arrhenius plot for ascorbic acid loss in orange juice at pH: 2.05.

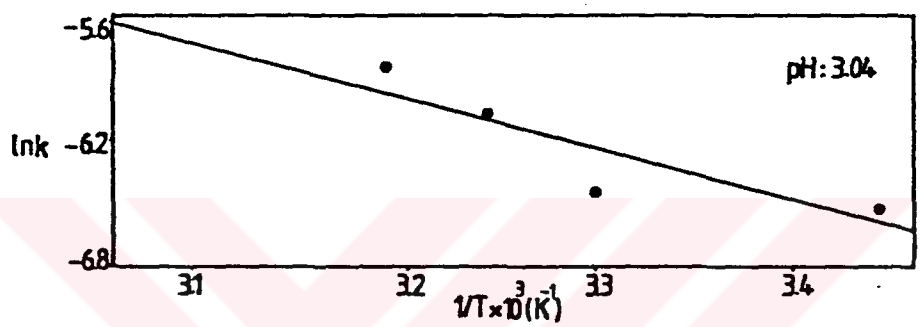


Figure 21. Arrhenius plot for ascorbic acid loss in orange juice at pH : 3.04.

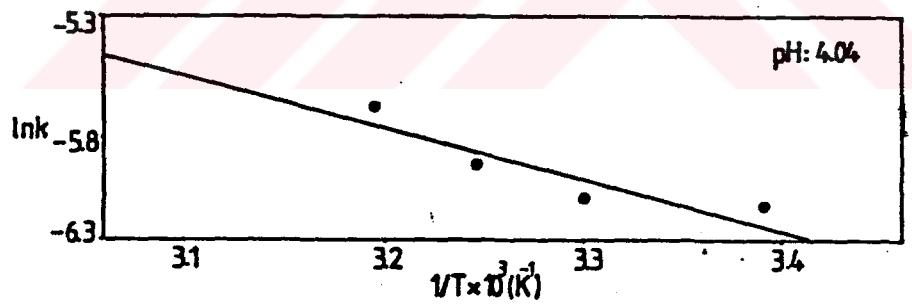


Figure 22. Arrhenius plot for ascorbic acid loss in orange juice at pH : 4.04.

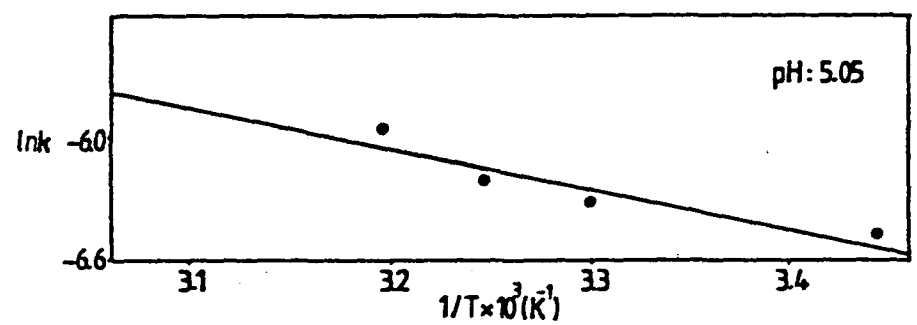


Figure 23. Arrhenius plot for ascorbic acid loss in orange juice at pH : 5.05.

Table 6. Kinetic parameters for the loss of ascorbic acid in orange juice at different pH values

pH	Activation energies (J/mol)	Intercept	Correlation Coefficient
2.05	99669.06	32.232	0.925
3.04	22591.63	2.750	-0.890
4.04	19961.33	1.929	-0.899
5.05	15770.16	0.0120	-0.934

and $$k = e \frac{kT}{h} e^{\Delta S^{\ddagger}/R} e^{-\Delta H^{\ddagger}/RT} \quad (25a)$$

Therefore the value of $\Delta H^{\ddagger}$ will be calculated from the slope and $\Delta S^{\ddagger}$ will be calculated from the intercept of the corresponding Arrhenius plots at each pH value. The harmonic mean of temperatures was chosen as the temperature to be used in Equations (26) and (25a) for the calculations both with orange juice and aqueous ascorbic acid. If the value of gas constant R is taken as 8.314 J/mol K, Equation 26 for $\Delta H^{\ddagger}$ becomes;

$$\Delta H^{\ddagger} \text{ (J/mol)} = E_{exp} - (2640.58)$$

for ascorbic acid solutions and

$$\Delta H^{\neq}\ (J/mol) = E_{exp} - (2508.17)$$

for orange juice solutions.

The intercept of an Arrhenius plot gives the value of ℓnA. Then, the equation for $\Delta S^{\neq}$ is

$$\Delta S^{\neq} = (\text{Intercept} - 1 - \ell n \frac{kT}{h}) \times R \qquad (33)$$

when the values of Boltzmann's constant and Planck's constant, the harmonic mean of temperatures and R = 8.314 J/mol K are put into the above equation , it becomes

$$\Delta S^{\neq} = (\text{Intercept} - 34.61) \times 8.314$$

for aqueous ascorbic acid solutions and

$$\Delta S^{\neq} = (\text{Intercept} - 34.56) \times 8.314$$

for orange juice solution.

Table 7 represents the values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ calculated for the loss of ascorbic acid in orange juice at four different initial pH conditions.

In order to calculate the isokinetic temperature, T_c , a

plot of $\Delta H^{\ddagger}$ versus $\Delta S^{\ddagger}$ should be prepared. This is given in Figure 24.As it is seen from Figure 24, there is an excellent correlation between $\Delta H/\Delta S$ data pairs at all pH conditions.The

Table 7. $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values calculated for the ascorbic acid loss in orange juice at different pH values

pH	$\Delta S^{\ddagger}$ (J/mol K)	$\Delta H^{\ddagger}$ (J/mol)
2.05	-19.39	91160.89
3.04	-264.49	20083.46
4.04	-271.33	17453.16
5.05	-287.26	13261.99

isokinetic temperature was given by the slope of this plot. It was calculated by the method of linear least squares. A value of 291.1 K was determined in this way as the isokinetic temperature, T_c , with a correlation coefficient of 0.999 for ascorbic acid loss in orange juice.

III.4.2. Results of Ascorbic Acid Loss in Aqueous Solutions

Treatment of the experimental data for the loss of ascorbic acid in aqueous solution at four different temperatures and each at four different pH values was exactly the same as explained in Section III.4.1. In this section in order to avoid repetition, only the necessary plots and

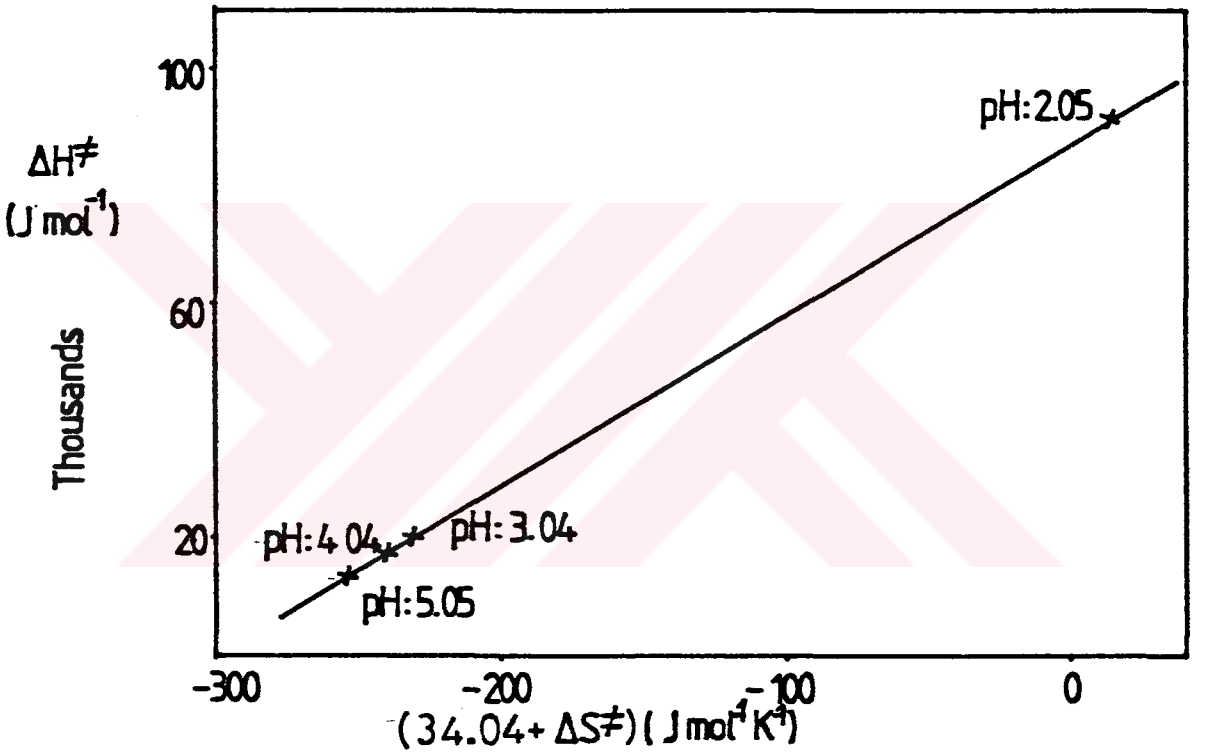


Figure 24. Enthalpy-entropy compensation plot for ascorbic acid degradation in orange juice.

figures will be represented . The pH values reported in figures and tables are obtained by taking the arithmetic average of pHs at all temperatures.

Table 5 includes the measured concentration of ascorbic acid at different reaction times and calculated rate constants.

Figures 25 to 39 are $-\ell nC_A$ versus time plots for the degradation of ascorbic acid in aqueous solution at each experimental condition.

Figure 40 represents the plot of rate constants versus pH for ascorbic acid loss in aqueous solution at different temperatures .

Figures 41-44 are the Arrhenius plots at all pH values. Calculated Arrhenius parameters are given in Table 8.

Table 8. Calculated Arrhenius parameters at each pH value for aqueous ascorbic acid solution

pH	Intercept	E_{exp}(J/mol)	Correlation Coefficient
2.38	6.690	36433.63	0.996
3.69	4.358	29533.98	0.997
4.90	3.328	26264.68	0.975
5.82	0.3812	18549.23	0.999

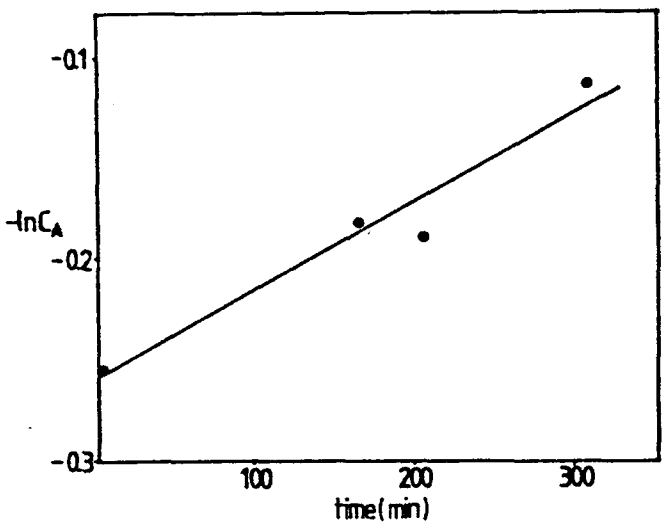


Figure 25. Ascorbic acid loss in aqueous solution at T=303 K, pH:2.44.

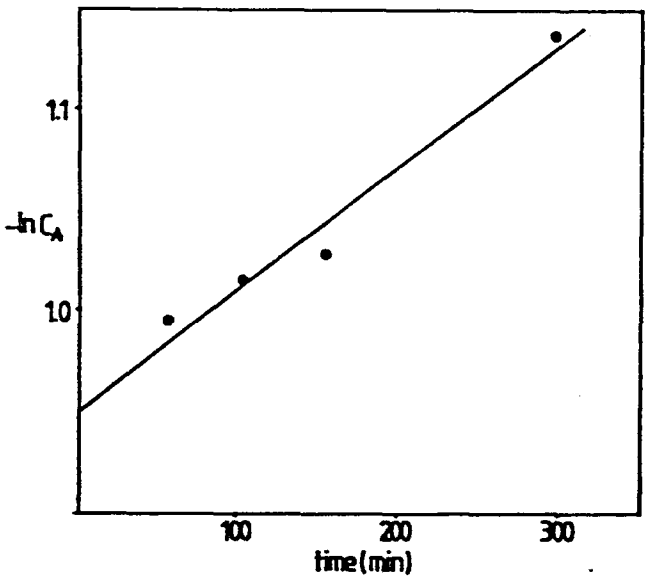


Figure 26. Ascorbic acid loss in aqueous solution at T=303 K, pH:3.76.

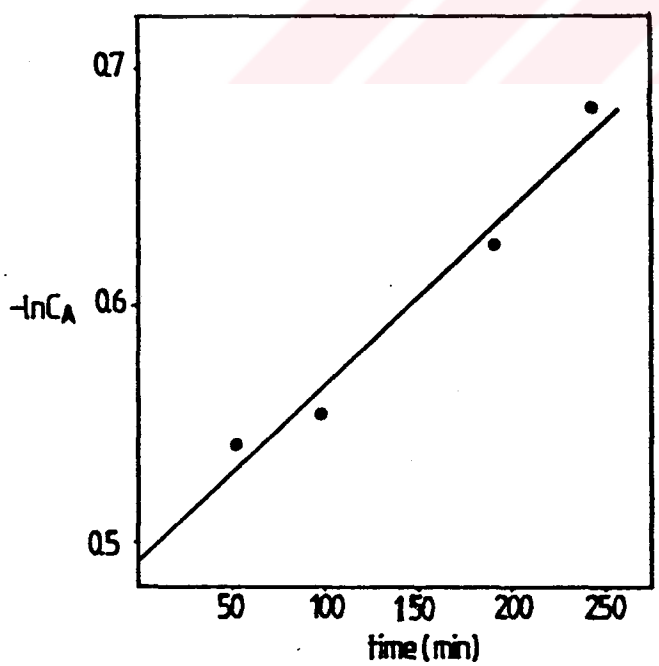


Figure 27. Ascorbic acid loss in aqueous solution at T=303 K, pH:4.93.

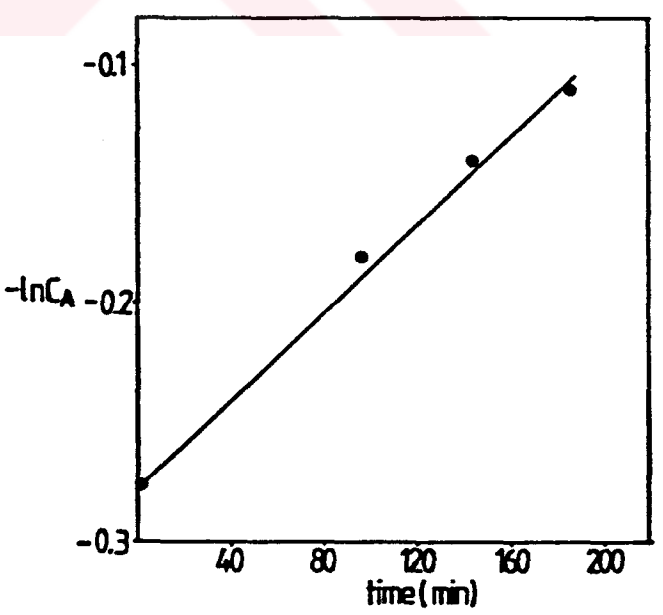


Figure 28. Ascorbic acid loss in aqueous solution at T=303 K, pH:5.72.

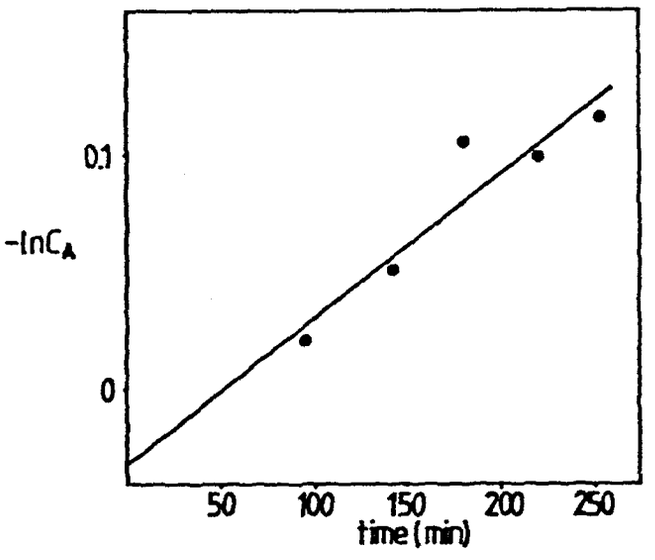


Figure 29. Ascorbic acid loss in aqueous solution at T= 313 K, pH:2 41.

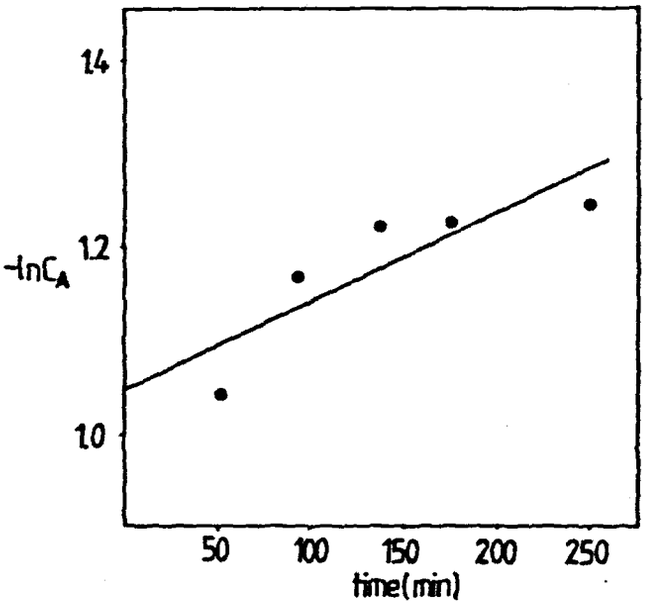


Figure 30. Ascorbic acid loss in aqueous solution at T=313 K, pH:3.68.

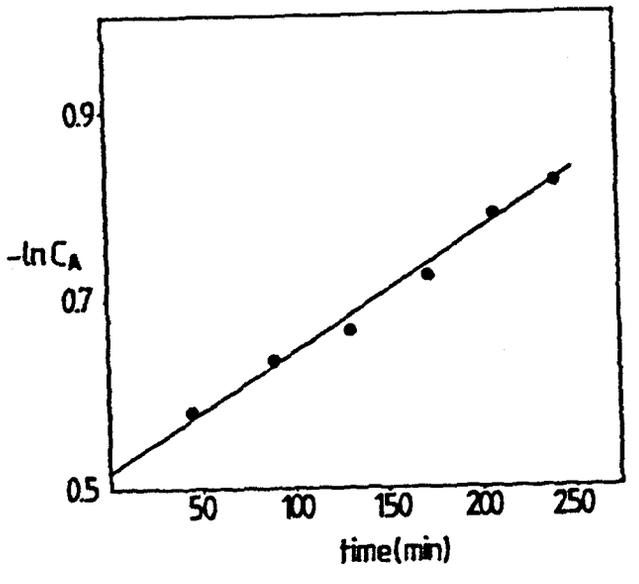


Figure 31. Ascorbic acid loss in aqueous solution at T=313 K, pH:4.91.

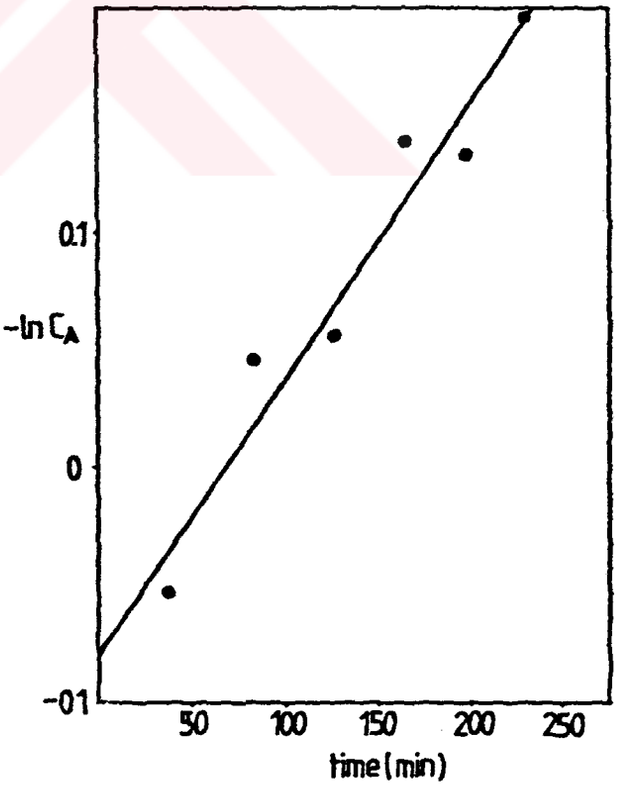


Figure 32. Ascorbic acid loss in aqueous solution at T=313 K, pH:5.90.

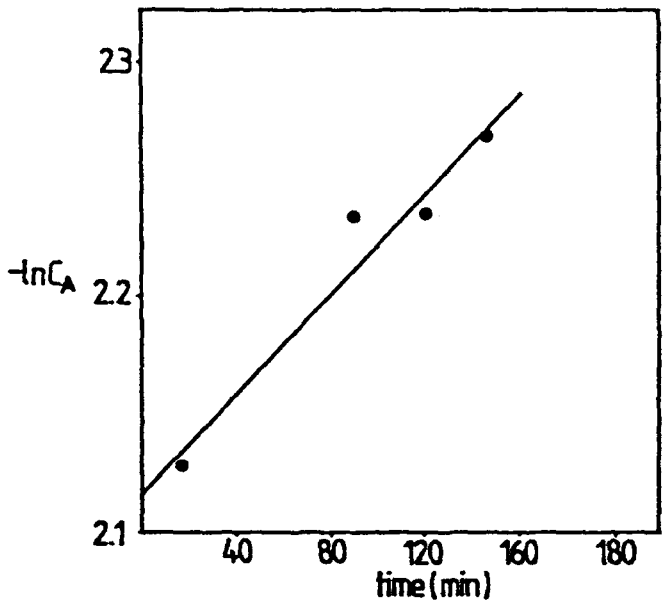


Figure 33. Ascorbic acid loss in aqueous solution at T=323 K, pH:2.28.

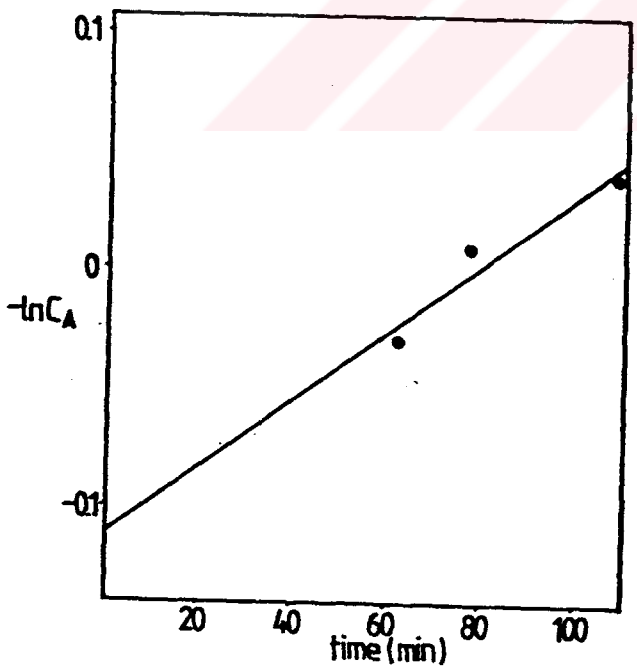


Figure 34. Ascorbic acid loss in aqueous solution at T= 323 K, pH:5.78.

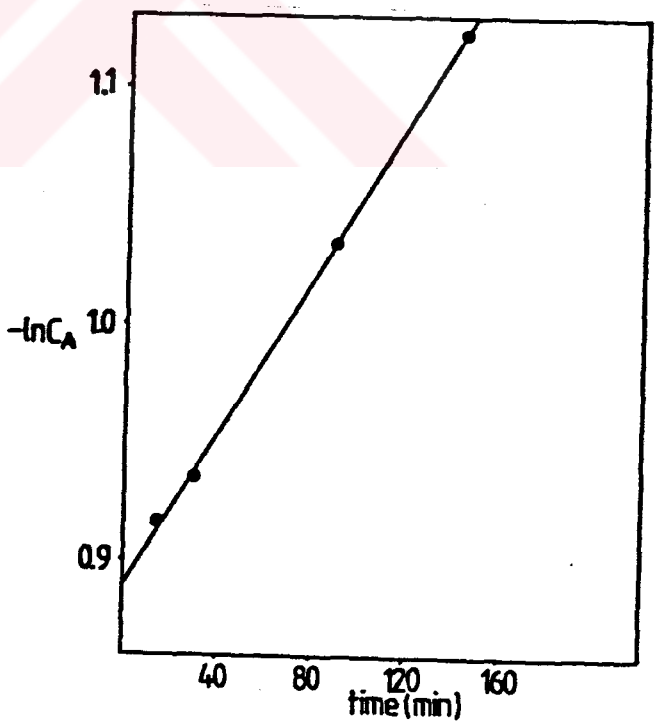


Figure 35. Ascorbic acid loss in aqueous solution at T= 323 K, pH:4.88.

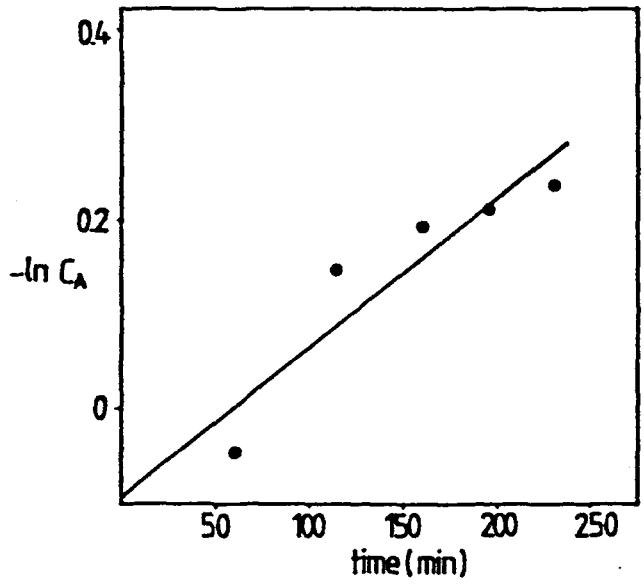


Figure 36. Ascorbic acid loss in aqueous solution at T= 333 K, pH:2.38.

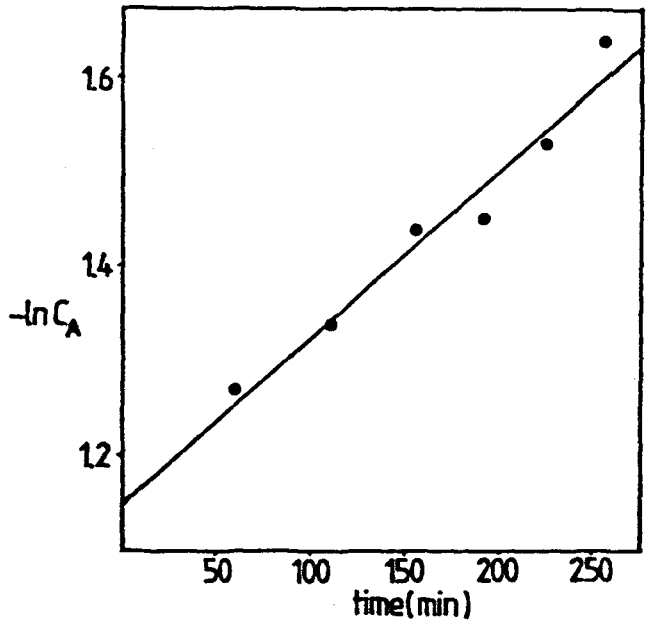


Figure 37. Ascorbic acid loss in aqueous solution at T= 333 K, pH:3.63.

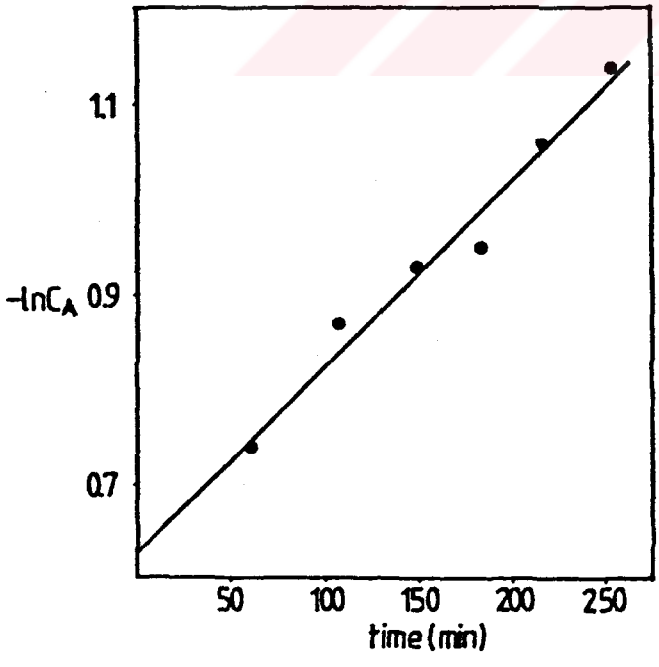


Figure 38. Ascorbic acid loss in aqueous solution at T=333 K, pH:4.88.

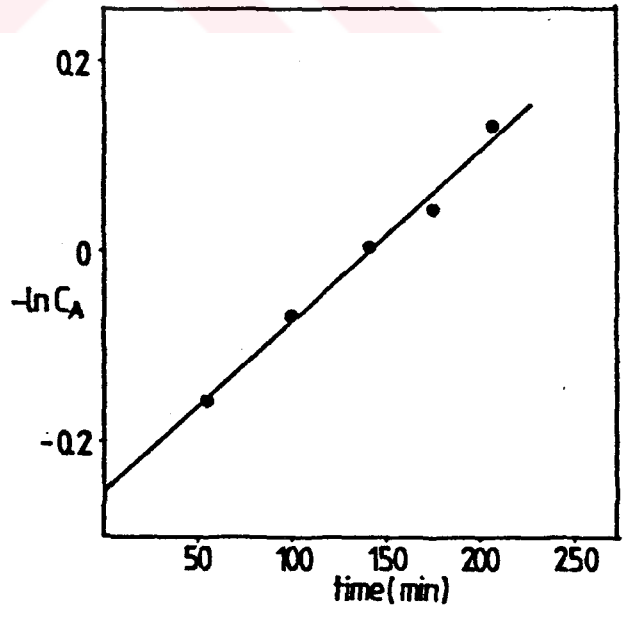


Figure 39. Ascorbic acid loss in aqueous solution at T=333 K, pH:5.88.

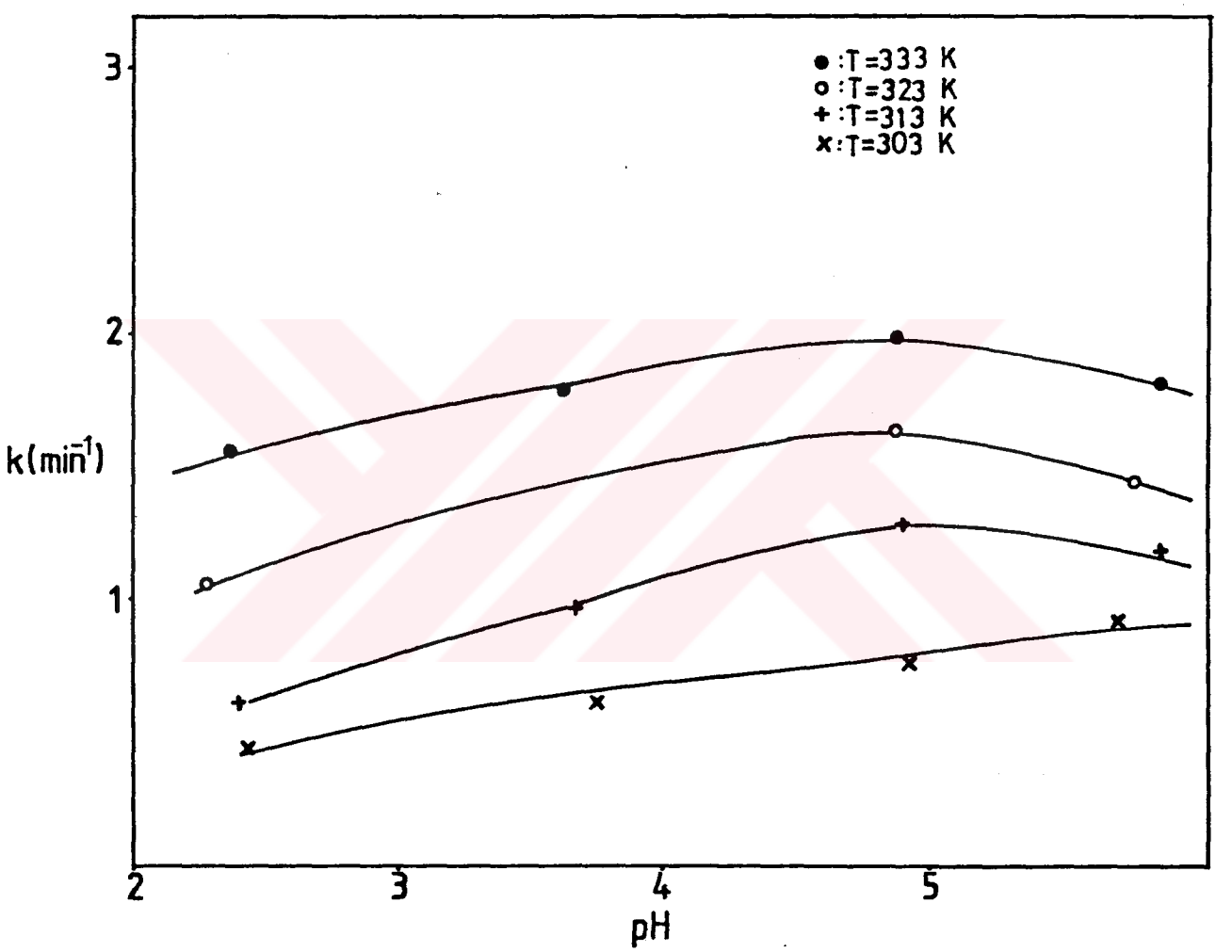


Figure 40. Plot of rate constants versus pH for ascorbic acid loss in aqueous solution at different temperatures.

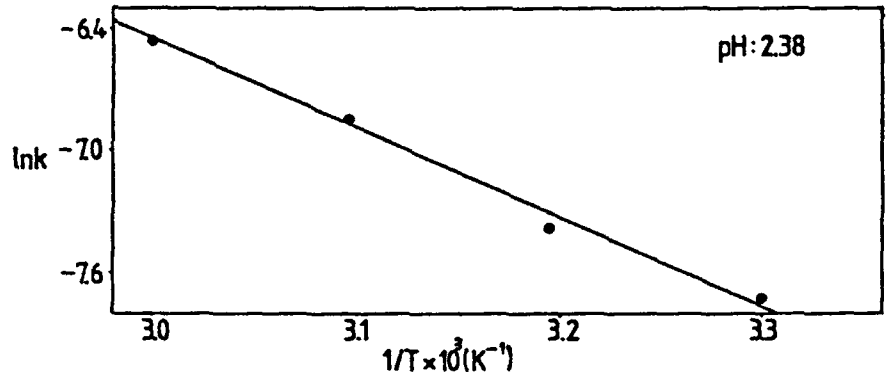


Figure 41. Arrhenius plot for loss of ascorbic acid in aqueous solution at pH : 2.38.

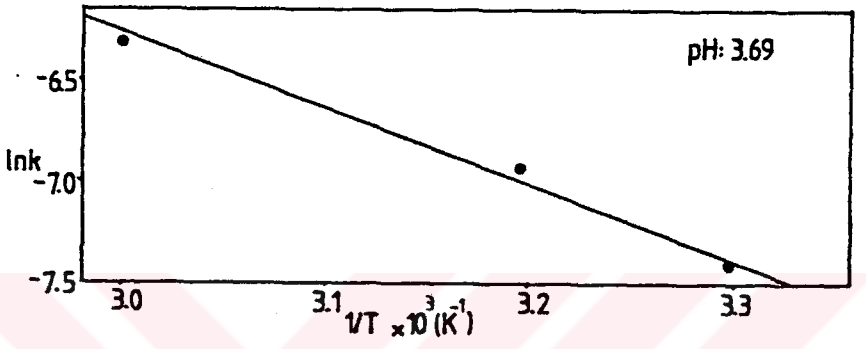


Figure 42. Arrhenius plot for loss of ascorbic acid in aqueous solution at pH : 3.69.

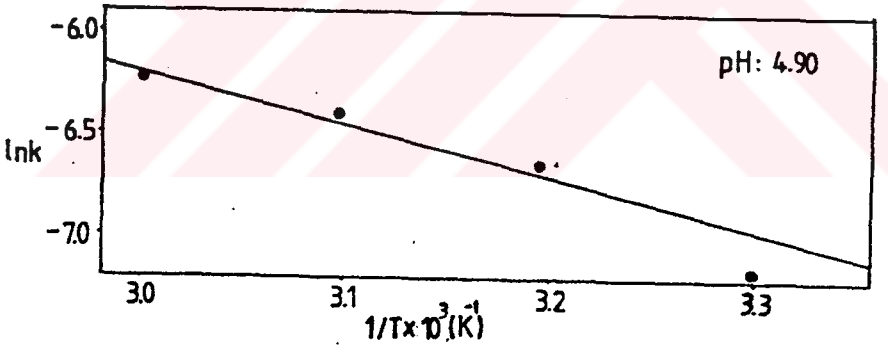


Figure 43. Arrhenius plot for loss of ascorbic acid in aqueous solution at pH : 4.90.

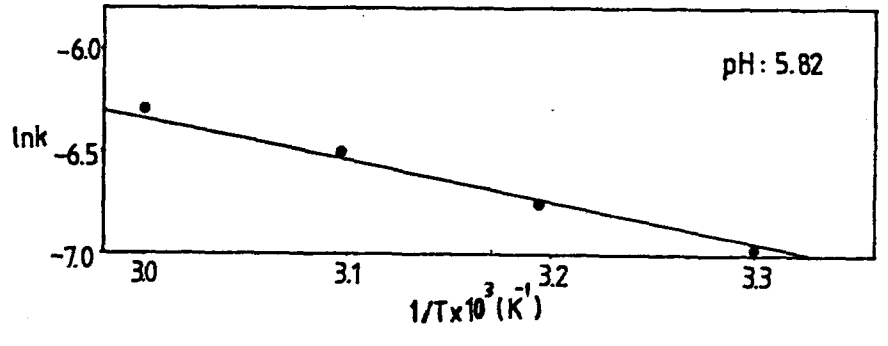


Figure 44. Arrhenius plot for loss of ascorbic acid in aqueous solution at pH : 5.82.

Table 9 represents the calculated $\Delta H^{\neq}$ and $\Delta S^{\neq}$ again at four different pH values.

Table 9. Calculated $\Delta H^{\neq}$ and $\Delta S^{\neq}$ values at each pH values for aqueous ascorbic acid solution

pH	$\Delta H^{\neq}$ (J/mol)	$\Delta S^{\neq}$(J/mol K)
2.38	33793.05	-232.17
3.69	26893.40	-251.56
4.90	23624.10	-260.12
5.82	15908.70	-284.62

Enthalpy-entropy compensation was shown in Figure 45. The isokinetic temperature, T_c , calculated from the slope of the line in Figure 45 for the loss of ascorbic acid in aqueous solution under different pH conditions was 341.2 K with the correlation coefficient of 0.999.

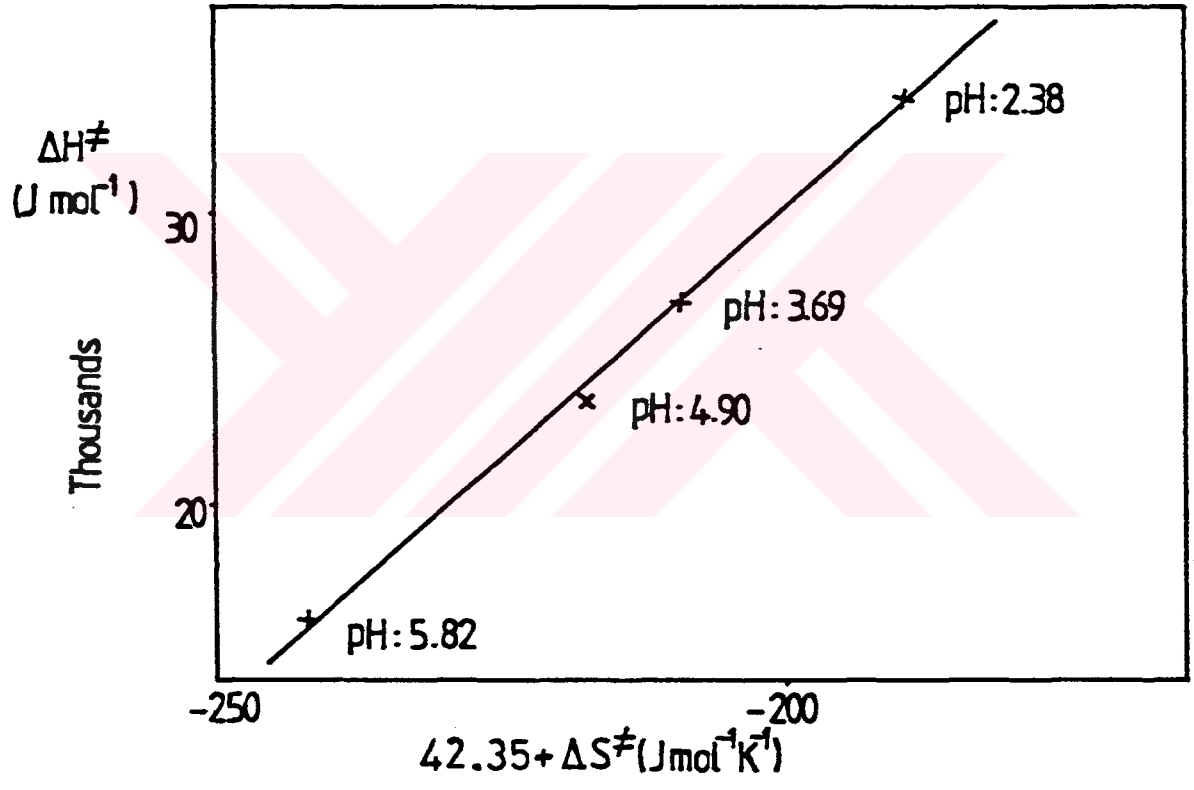


Figure 45. Enthalpy-entropy compensation plot for ascorbic acid degradation in aqueous solution.

CHAPTER IV

DISCUSSION

Experimentally determined activation energies for the loss of ascorbic acid in aqueous solution range between 18.5 and 36.4 kJ/mol and in orange juice between 15.8 and 99.7 kJ/mol depending on the pH of systems. It is difficult to find direct comparisons in the literature for this purpose, however, they seem to be in reasonable agreement with other reported values [15,32,16,11,6].

The values of activation enthalpy for ascorbic acid loss in aqueous solution between the pH values 2.38 and 5.82 are in the range of 15.9 and 33.84 kJ/mol and in orange juice between pH 2.05-5.05 are in the range of 13.3 and 91.2 kJ/mol. On the other hand, activation entropies calculated in this study were between -19.39 and -287.26 J/mol K for orange juice and between -232.17 and -284.62 J/mol K for aqueous solutions.No study in the literature reports these thermodynamic quantities either in aqueous solution alone or in food systems.Labuza [6] gave a table in which the calculated thermodynamic quantities were presented from the studies of other workers. Although the experimental conditions are not exactly same (e.g., reaction rates were determined with changing water activity, at different temperature ranges),

the results obtained in this study are quite comparable to the calculated values in Labuza's paper.

Enthalpy-entropy compensation have been one of the most widely discussed phenomena in recent years. Since the presence of a linear relation between enthalpy and entropy is observed in a variety of systems such as organic chemistry, protein-enzyme-water reactions, microbial death and in food deterioration, it is very popular among scientists. Although it is so popular, it has not yet been completely understood.

The study of enthalpy-entropy compensation requires a large number of experimental data with great accuracy. In this study, enthalpy-entropy plots showed excellent linearity with correlation coefficients very close to 1. There are some methods to test the results obtained from such studies. These were discussed and applied to the results of the present study below:

1) All ℓnk versus E_{exp} plots should be linear.

The basis of this test is in the use of Arrhenius expression itself. When this test was applied to the results of the present study, acceptable linearity was observed especially in ℓnk versus E_{exp} plots for the ascorbic acid loss in aqueous solution. These plots are shown in Figures 46 and 47. The correlation coefficients included in plots indicate that the relation between ℓnk and E_{exp} at each temperature might be assumed to be linear.

2) All ℓnk versus $1/T$ plots should intersect at $1/T_c$.

If a typical activation enthalpy versus activation

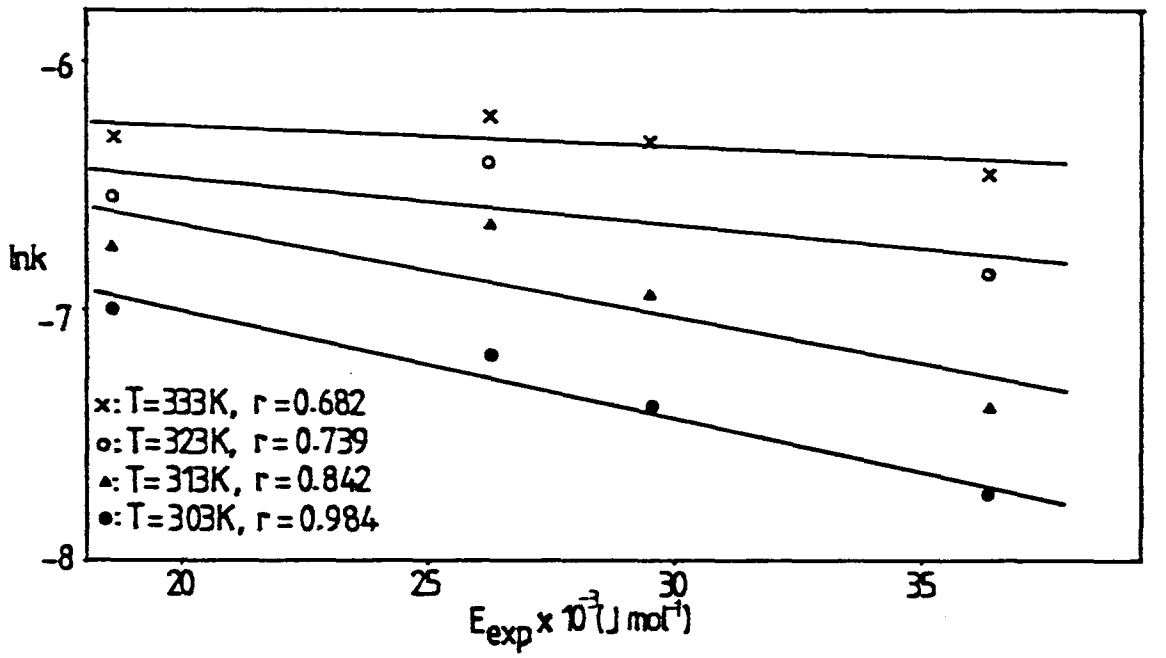


Figure 46. ℓnk versus E_{exp} plots for the ascorbic acid loss in aqueous solution.

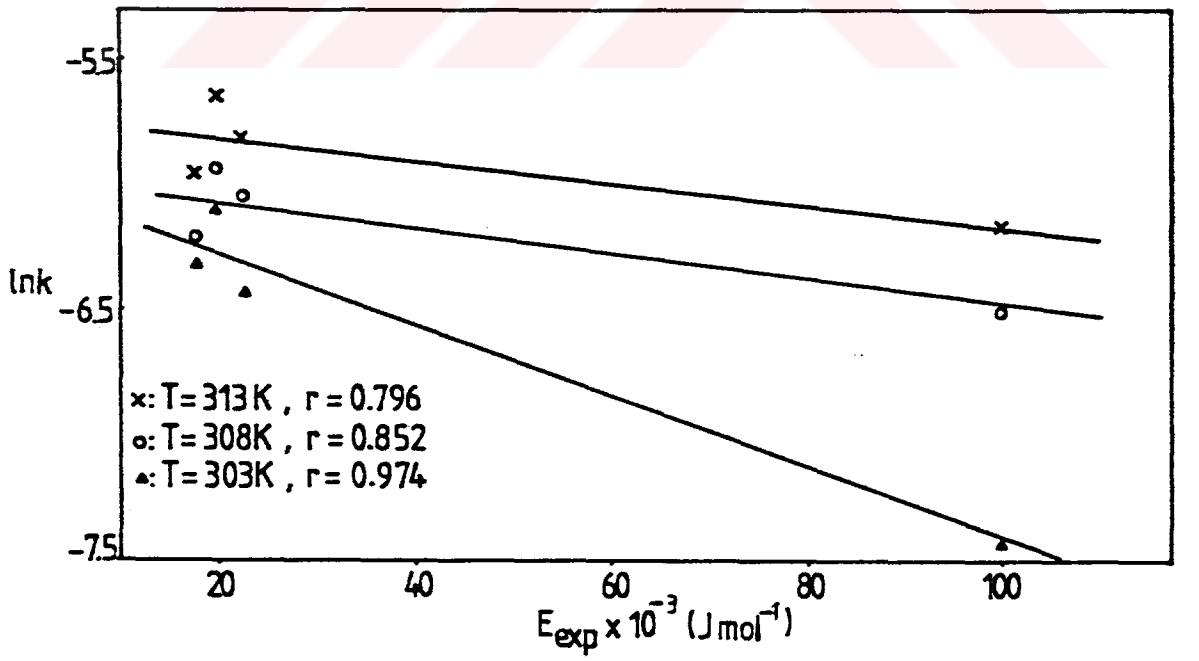
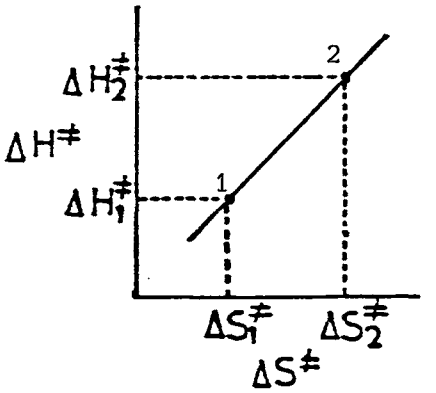


Figure 47. ℓnk versus E_{exp} plots for the ascorbic acid loss in orange juice

entropy plot as shown below is prepared,



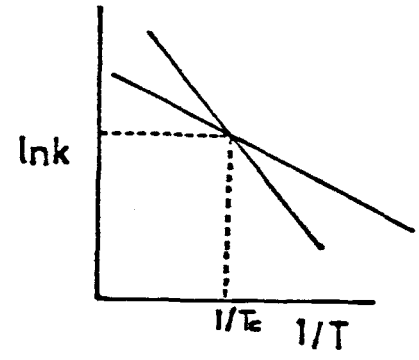
the slope of the line calculated between two data pair points (1 and 2) is equal to

$$\text{Slope} = \frac{\Delta H_2^{\ddagger} - \Delta H_1^{\ddagger}}{\Delta S_2^{\ddagger} - \Delta S_1^{\ddagger}} = T_c$$

Using the equations $\Delta H^{\ddagger} = E_{exp} - RT$ and $\Delta S^{\ddagger} = R\ell nA - R\ell ne\frac{kT}{h}$, it can be shown that,

$$T_c = \frac{(E_{exp})_2 - (E_{exp})_1}{R[(\ell nA)_2 - (\ell nA)_1]}$$

On the other hand, when all the ℓnk values at different pHs are plotted with respect to 1/T, as shown below, at the intersection point, all lines will have the same ℓnk value. By equating the Arrhenius expressions for a pair of lines, the value of the intersection point on 1/T axis can be obtained as ;



$$\frac{1}{T} = \frac{(\ell nA)_2 - (\ell nA)_1}{\frac{(E_{exp})_2}{R} - \frac{(E_{exp})_1}{R}}$$

which is equal to $1/T_c$. When this test was applied to the results of present study, plots shown in Figures 48 and 49 were obtained.

For the calculation of $1/T_c$ values from these plots, the values of ℓnk and 1/T at the intersection point of two lines were determined by the method of least squares and results were summarized in Table 10 for aqueous ascorbic acid solutions.

As can be seen in Table 10, the calculated T_c values from this test ranges between 315 and 382 K. T_c derived for ascorbic acid loss from aqueous solutions was 341.2 K. Therefore, if the mean of T_c values from Table 10 is taken, which is 346.9 K, it will be concluded that the value of

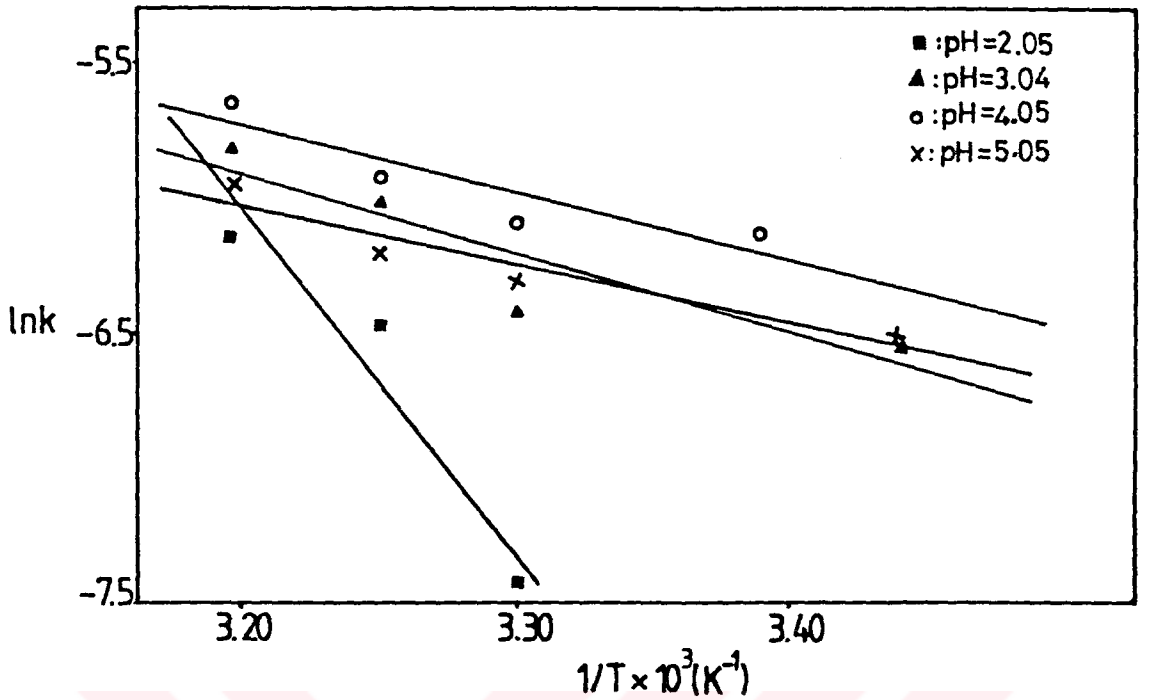


Figure 48, ℓnk versus 1/T plots for the ascorbic acid loss in orange juice.

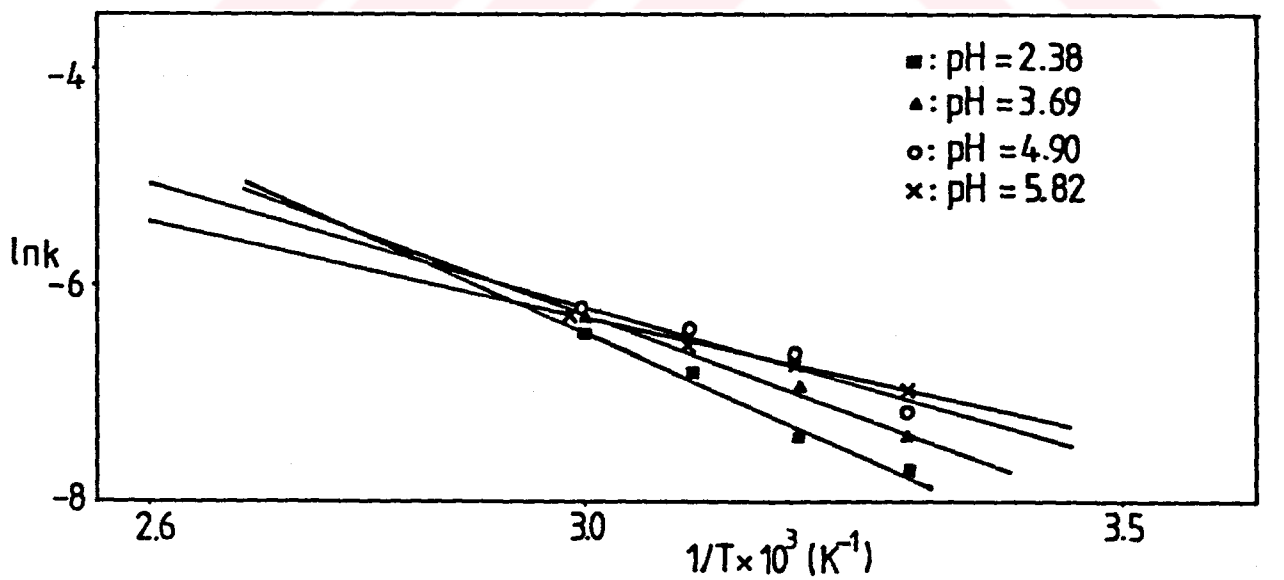


Figure 49, ℓnk versus 1/T plots for the ascorbic acid loss in aqueous solution.

Table 10. ℓnk and 1/T values at intersection points of lines from aqueous ascorbic acid studies

Intersecting lines with pHs	Intersection points		
	ℓnk	1/T (K^{-1})	T_c, (K)
5.82-4.90	-6.70	0.00318	314.9
5.82-3.69	-6.33	0.00301	322.3
5.82-2.38	-6.16	0.00293	341.0
4.90-3.69	-4.94	0.00262	382.0
4.90-2.38	-5.35	0.00275	363.9
3.69-2.38	-5.62	0.00281	355.9

Table 11. ℓnk and 1/T values at intersection points of lines from orange juice studies

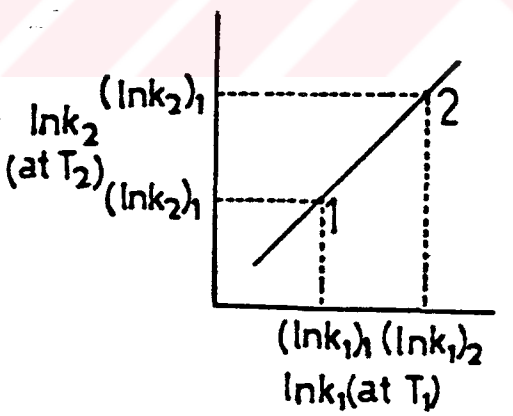
Intersection lines with pHs	Intersection points		
	ℓnk	1/T (K^{-1})	T_c, (K)
5.05-4.04	-7.20	0.00318	262.99
5.05-3.04	-6.32	0.00333	299.62
5.05-2.05	-6.04	0.00319	313.20
4.04-3.04	-4.31	0.00259	385.10
4.04-2.05	-5.66	0.00316	316.40
3.04-2.05	-5.89	0.00318	314.50

compensation temperature obtained from activation enthalpy-activation entropy is supported by this test.

Table 11 represents the results of this test when applied to the loss of ascorbic acid in orange juice.
The mean of T_c values, when calculated from data in Table 11 appears to be 311.4 K. T_c value from activation enthalpy-activation entropy plot for ascorbic acid loss in orange juice was 291.1 K. Although the two T_c values are not very close to each other, they are not far away enough to conclude that this test rejects the value of T_c obtained from thermodynamic parameters.

3) Plots of ℓnk_2 versus ℓnk_1 at T_2 and T_1 should give the value of T_c.

When a typical plot of ℓnk_2 versus ℓnk_1 at corresponding temperatures T_2 and T_1 is prepared as shown below,



the slope of the line is equal to

$$\text{Slope} = \frac{(\ell nk_2)_2 - (\ell nk_2)_1}{(\ell nk_1)_2 - (\ell nk_1)_1}$$

By writing the Arrhenius expressions for the rate constants and using the equation of $T_c, (E_{exp})_2 - (E_{exp})_1 / R[(\ell nA)_2 - (\ell nA)_1]$ the equation given below is obtained for the slope

$$\text{Slope} = \left(\frac{T_2 - T_1}{T_1 - T_c}\right)\left(\frac{T_1}{T_2}\right)$$

Data from the loss of ascorbic acid in aqueous solution and in orange juice were treated according to this test and plots shown in Figures 50 and 51 were prepared. Table 12 represents the T_c values and correlation coefficients obtained from this test.

Linearity of the lines in Figures 50 and 51 are most of the time sufficient to calculate their slopes. Table 12 includes a large range of T_c values whose mean gives T_c as 283 K for aqueous ascorbic acid solutions and 281.1 K for orange juice solutions. The agreement of T_c values from the test results and from activation enthalpy-activation entropy plots for the loss of ascorbic acid in aqueous solutions may not be claimed to be as good as in the case of orange juice systems, however, it should be mentioned that this test is mostly referred to be a very difficult test, Lumry and Rajender [4] indicated that many of the reactions which showed true chemical compensation would not pass this test.

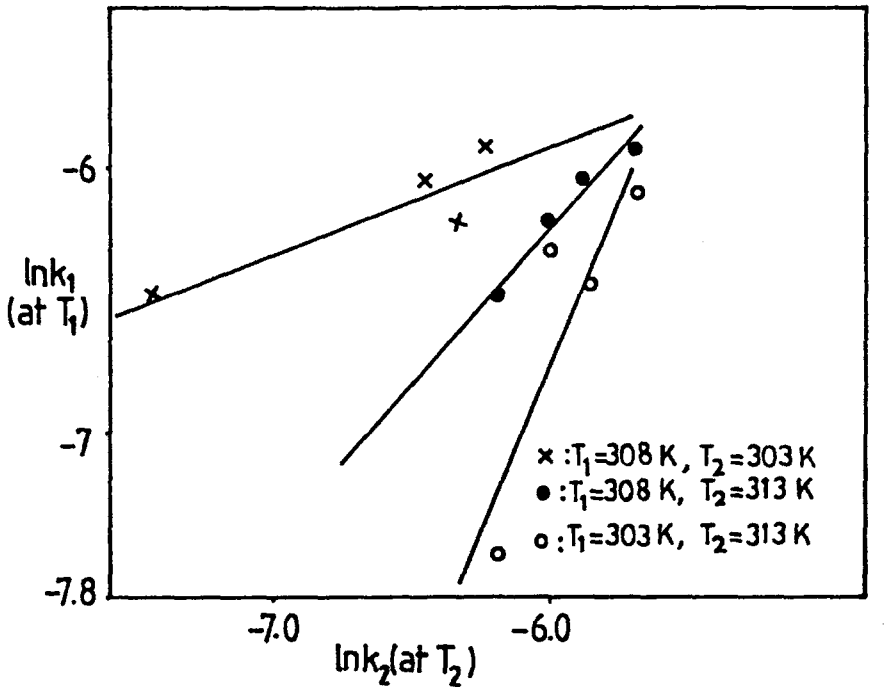


Figure 50. Plot of ℓnk_2 versus ℓnk_1 at T_2 and T_1 for the loss of ascorbic acid in orange juice.

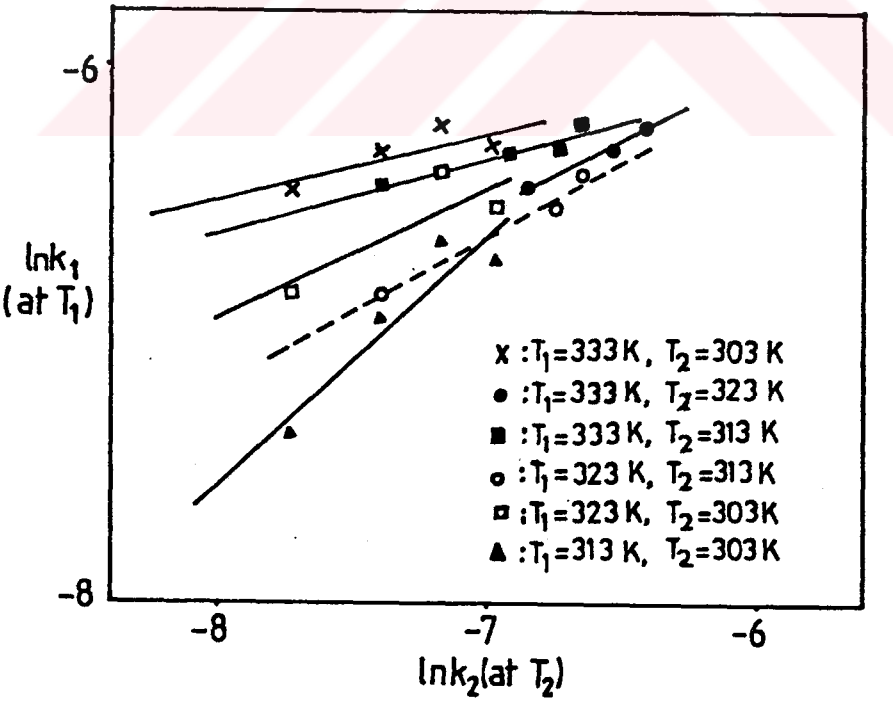


Figure 51. Plot of ℓnk_2 versus ℓnk_1 at T_2 and T_1 for the loss of ascorbic acid in aqueous solution.

Table 12. T_c values from ℓnk_2 (at T_2) versus ℓnk_1 (at T_1) plots

T_1 (K)	T_2 (K)	T_c (K)	r
Aqueous Ascorbic acid solutions			
333	323	294.3	0.803
333	323	312.1	0.998
333	313	305.4	0.969
323	313	301.7	0.984
323	303	285.9	0.851
313	303	198.5	0.925
Orange juice solutions			
308	303	300.2	0.918
308	313	246.9	0.992
303	313	296.1	0.889

Aside from the tests discussed upto now, some scientists discuss the occurrence of the compensation between enthalpy and entropy from the statistical point of view.

Leffler [33], Kemeny and Rosenberg [5], Boon [34], Harris [35] and particularly Krug, Hunter and Grieger [36,37,38] discussed this phenomenon in detail,statistically. It was suggested that the occurrence of enthalpy-entropy compensation in many reactions, if not all, did not show the true chemical compensation. Krug and his friends say that the use of linear least squares method for the calculation of T_c

from enthalpy-entropy plots is not suitable since both of enthalpy and entropy are not independent variables.

They believe that the fit of enthalpy-entropy data to a straight line is fortuitous, because when data are plotted in the usual way, the true functional dependence, if any , is usually masked by a dominant statistical compensation pattern that arises from experimental errors. Therefore, the observed distribution of data points along straight lines in the enthalpy-entropy plane is more often due to propagation of measurement errors than to chemical variation.

The most common argument among the scientists is the fact that, in most of the analysis, T_c calculated from enthalpy-entropy plots appears to be equal or very close to the harmonic mean of experimental temperatures. Harmonic mean of temperatures, T_{hm}, which is equal to $n/\Sigma(1/T)$, was shown to be equal to the slope of the statistical error line in many of the studies. In these studies, in which, distribution of experimental errors create a statistical compensation line instead of a chemical compensation line, the correlation coefficient was also shown to be very close to 1. Especially, if the temperature range is narrow, the correlation coefficient of the statistical error line is exactly equal to 1. Therefore, Krug and his friends [36,37] suggested that if the correlation coefficient of the enthalpy-entropy compensation line is calculated to be very close to 1, one should suspect the occurrence of a statistical error line rather than that of a true compensation.

T_{hm} in the present work is 317.6 K for the studies in aqueous ascorbic acid solutions and 301.7 K for the experiments performed with orange juice. T_c calculated from enthalpy-entropy plots for aqueous ascorbic acid solutions is 341.2 K and it is 291.1 K for orange juice systems.Therefore, comparison suggests that for both systems, T_{hm} and T_c are not equal to each other, and hence the possibility of real chemical compensation might be present.

However,the correlation coefficients of enthalpy-entropy plots in this work are both 0.999. According to Krug's discussion, then, the statistical error compensation line might be superimposing on the chemical compensation line.Krug and his friends [36] suggest a testing method for such cases in order to separate the effect of statistical compensation pattern from the chemical one. The basis of their test is to show the chemical compensation between two independent variables instead of between two dependent variables. In enthalpy-entropy plots, since both of the variables are obtained from the slope and intercept of the same Arrhenius plot, their errors are not independent from each other statistically. Thus, they argue that, if there is a linear relationship between enthalpy and entropy by the equation, $\Delta H=T_c\Delta S + \Delta G_{T_c}$ this must be consistent with the thermodynamic Gibbs equation $\Delta G=\Delta H - T\Delta S$ and a linear relationship should also exist between enthalpy and free energy evaluated at T_{hm}. The relation between ΔH and ΔG was given as

$$\Delta H = \gamma\Delta G + (1-\gamma)\ \Delta G_{T_c}$$

It can be shown from the two enthalpy equations above that, $\gamma = 1/1-(T_{hm}/T_c)$. Here, γ is the slope of enthalpy-free energy plot, T_c is the isokinetic temperature and T_{hm} is the harmonic mean of the temperatures. They suggest that both of the activation free energy and activation enthalpy should be evaluated at T_{hm}.

In order to plot activation enthalpy with respect to activation free energy, they write the linearized Arrhenius equation in another equivalent form as shown below :

$$\ell nk = \left(\ell nA - \frac{E}{R\,T_{hm}}\right) - \frac{E}{R}\left(\frac{1}{T} - \frac{1}{T_{hm}}\right)$$

Therefore, they suggest that if ℓnk values are plotted with respect to $\frac{1}{T} - \frac{1}{T_{hm}}$, activation enthalpy will be obtained from the slope of the line as before and the intercept will be a measure of $\Delta G^{\neq}$ evaluated at T_{hm}. Thus,

$$\Delta G^{\neq} = -RT_{hm}\left(\ell nA - \frac{E}{R\,T_{hm}}\right) + R\,T_{hm}\,\ell n\left(e\,\frac{\underline{k}\,T_{hm}}{h}\right) - R\,T_{hm}$$

intercept

In this way, the errors of activation enthalpy and

activation free energy were made uncorrelated and the statistical correlation coefficient was proven to be equal to zero. Hence, a high correlation coefficient observed in activation enthalpy versus activation free energy plots will be the indication of true chemical compensation.

In order to apply Krug's this test to the results of the present work, all data were retreated by preparing ℓnk versus $\frac{1}{T} - \frac{1}{T_{hm}}$ plots at each pH value for the loss of ascorbic acid in both aqueous solution and in orange juice. For the purpose of clarity, these plots will not be shown in the text. Table 13 represents activation enthalpy calculated from the slope and activation free energy calculated from the intercepts of new Arrhenius plots, both at T_{hm}.

Final step is to plot the values of activation enthalpy versus activation free energy. These plots are shown in Figures 52 and 53. The correlation coefficients of the lines are 0.881 for aqueous ascorbic acid solutions and 0.981 for orange juice systems. These correlation coefficients now are the correlation coefficients of only chemical compensation lines free from statistical error lines. Therefore, these high correlation coefficients prove that the compensation observed in this study is true chemical compensation. Isokinetic temperatures were calculated from the slope of lines by using the equation

$$\text{Slope} = \frac{1}{1 - \frac{T_{hm}}{T_c}}$$

Table 13. Activation enthalpies and activation free energies obtained as a result of the application of Krug's test to ascorbic acid loss in both aqueous solution and in orange juice.

pH	$\Delta H^{\neq}$ (J/mol)	$\Delta G^{\neq}$ (J/mol)
	Aqueous Ascorbic Acid Solution	
2.83	33793.04	69995.49
3.69	26893.40	70737.10
4.90	23624.12	71288.56
5.82	15908.68	71221.77
	Orange Juice	
2.05	97131.09	65360.24
3.04	20086.33	68490.61
4.04	17459.28	69060.25
5.05	13217.78	68441.43

and T_c obtained were 291.1K for ascorbic acid loss in aqueous solution and 289.4 K for ascorbic acid loss in orange juices.

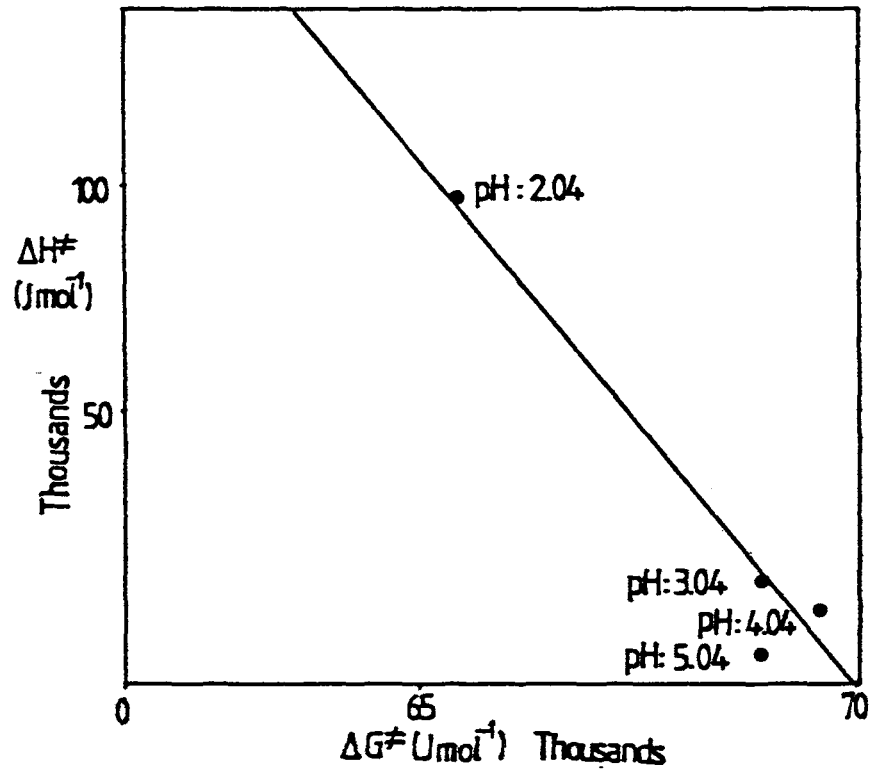


Figure 52. $\Delta H^{\neq}$ versus $\Delta G^{\neq}$ plots for loss of ascorbic acid in orange juice

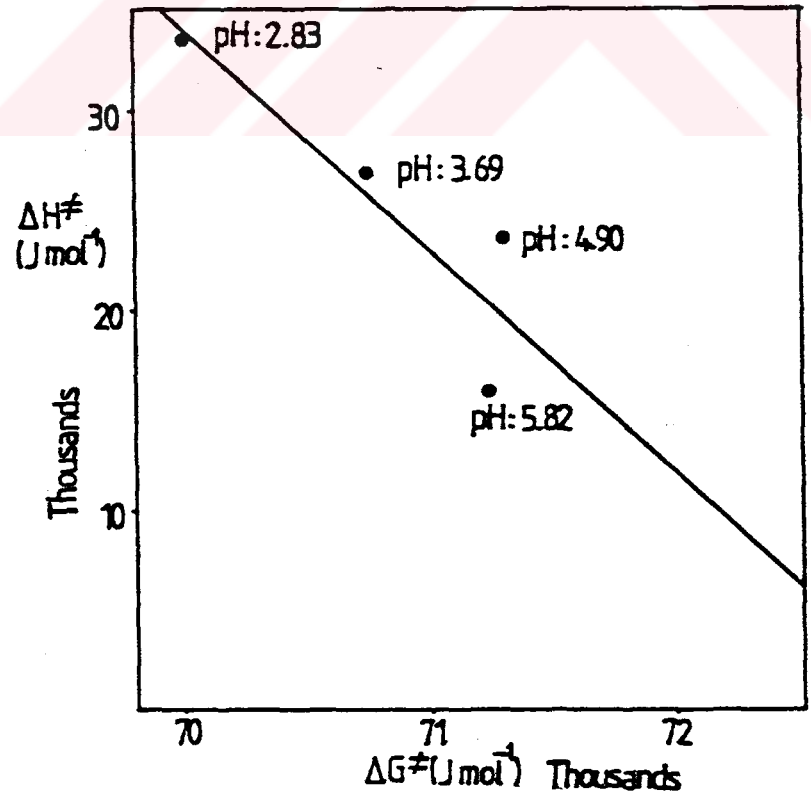


Figure 53. $\Delta H^{\neq}$ versus $\Delta G^{\neq}$ plots for loss of ascorbic acid in aqueous solution.

When the corresponding T_c values from activation enthalpy-entropy plots and from activation enthalpy-free energy plots are compared, T_c obtained from the later plot seems to be low for aqueous ascorbic acid solutions whereas T_cs obtained from both plots are very close to each other for orange juice studies. It is difficult to explain this result, however both T_c s may still be accepted to have reasonable values, because they are not out of range very much.

Labuza's calculations from the data of other scientists indicated a T_c of 350 K for ascorbic acid loss in aqueous ascorbic acid solutions and 274 and 281 K for ascorbic acid loss in various food systems from $\Delta H - \Delta S$ plots. Therefore, the isokinetic temperatures obtained from this study for both systems are in reasonable agreement with this limited number of data from literature. The T_c s obtained from enthalpy-free energy plots for ascorbic acid loss in orange juice and in aqueous ascorbic acid solutions are almost equal to each other unexpectedly. These activation enthalpy-activation free energy plots however should be considered as testing plots for the separation of statistical and chemical compensation lines. Krug [37] shows the plots of activation enthalpy-activation free energy with points which are impossible to put on a line. These plots then, indicate the loss of chemical compensation in contrary to the results of the present work.

Another suggestion to be sure about the existence of a chemical compensation between enthalpy of activation and

entropy of activation is the determination of these variables by independent methods, for example, determination of activation entropy from Arrhenius plots and determination of activation enthalpy by calorimetric methods. This seems to be the best method for future analysis of chemical compensation in food reactions.

CHAPTER V

CONCLUSIONS

1. The rates of ascorbic acid loss in orange juice and in aqueous ascorbic acid solution at different temperature and pH conditions was followed by HPLC method using Novapak C_{18} column and the mobile phase of (20:80) methanol/water adjusted to pH 5-5.5.

2. The rate of ascorbic acid loss in orange juice increases towards pH 4 and then decreases towards pH 5.

3. The rate of ascorbic acid loss in aqueous solution raises upto pH 5, and then decreases slightly towards pH 6.

4. Activation energies calculated from Arrhenius plots for each different pH conditions were in the range of 15.8 and 99.7 kJ/mol for orange juice and in the range of 18.5 and 36.4 kJ/mol for aqueous ascorbic acid solutions.

5. Activation enthalpies as calculated from the activation energies for the loss ascorbic acid were in the range of 13.3 and 91.2 kJ mol^{-1} for orange juice, and were in the range of 15.9 and 33.84 kJ mol^{-1} for aqueous solutions.

6. Activation entropies were calculated from the intercept of Arrhenius plots. They were ranging from -19.39 to -287.26 J/mol K for orange juice and from -232.17 to -284.62 J/mol K for aqueous solutions.

7. The plot of enthalpy-entropy data pairs at each different pH conditions gave a straight line whose slope, isokinetic temperature, was found to be 291.1 K for the loss of ascorbic acid in orange juice and 341.2 K for the loss of ascorbic acid in aqueous solutions.

8. Several test methods developed by other workers were applied to understand whether true chemical compensation was observed or not. Most of the test methods indicated the presence of true chemical compensation in the present work for the loss of ascorbic acid in both aqueous solution and in orange juice.

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