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# KINETIC ANALYSIS OF THE LOSS OF SOME B VITAMINS DURING THE COOKING OF MACARONI

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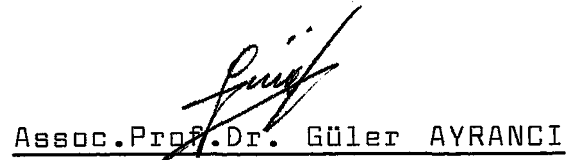
We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Food Engineering.



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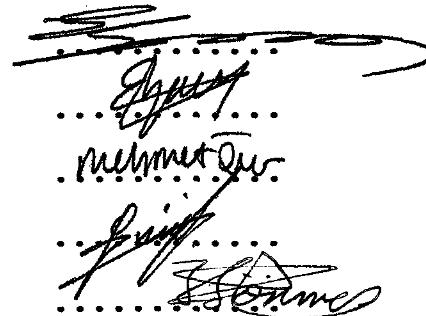

# ABSTRACT

## KINETIC ANALYSIS OF THE LOSS OF SOME B VITAMINS DURING THE COOKING OF MACARONI

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M.Sc. in Food Engineering

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A method was developed for the simultaneous determination of thiamin, niacinamide and riboflavin in macaroni by High Pressure Liquid Chromatography (HPLC). The kinetics of losses of these vitamins during cooking were studied.

By applying two-step linear least square technique, the rate constants and the Arrhenius parameters (activation energies and frequency factors) of these vitamins were calculated. The activation for the losses of thiamin, niacinamide and riboflavin were found to be 25, 22 and 11 $kJ.mol^{-1}$, respectively. The values of entropy of activation, enthalpy of activation and Gibbs free energy of activation were also calculated.

By considering the characteristic stabilities of these vitamins in pure forms, the factors effective for the extraction of these vitamins from macaroni and the proces of diffusion in water, it was concluded that the

activation energies obtained in this study were representing the activation energies for the leaching processes of these vitamins into water. Therefore, thiamin appears to be the vitamin which is most difficult to leach into water solution during cooking of macaroni. However, when the experimental data are analyzed for percent losses of the vitamins, it is clear that more than 40-50 % of losses for each vitamin occur during cooking of macaroni at about 363 K. Therefore, it seems that the cooking water should not be discarded after cooking of macaroni in order to have a more nutritional diet.

**Key words:** HPLC, thiamin, niacin, riboflavin, losses of vitamins, kinetic analyses and Arrhenius parameters.

# ÖZET

## MAKARNANIN PİŞİRİLMESİ SIRASINDA BAZI B VİTAMİNLERİN KAYBININ KİNETİC ANALİZİ

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Makarnanın içindeki tiamin, niasinamid ve riboflavin miktarlarını aynı anda ölçmek için Yüksek Basınçlı Sıvı Kromatografisi kullanıldı. Değişik sıcaklıklarda bu vitaminlerin pişme sırasında kaybının kinetiği incelendi ve Arrhenius parametreleri elde edildi. Bu hesaplamalar sırasında en küçük kareler doğrusu metodu kullanıldı. Bu metotla önce reaksiyon sabiti, daha sonra Arrhenius parametreleri (aktivasyon enerjileri ve frekans faktörleri) hesaplandı.

Aktivasyon enerjisi, tiamin, niasinamid ve riboflavin için sırası ile 25, 22 ve 11 $kJ.mol^{-1}$ olarak bulundu. Her bir vitamin için, aktivasyon entalpisi, entropisi ve Gibbs serbest enerjisi de hesaplandı.

Bu vitaminlerin saf haldeki stabiletelerini, makarnadan suya geçmelerini etkileyen faktörleri ve sudaki difüzyon prosesini düşünerek, bu çalışmada elde edilen aktivasyon enerjilerinin, vitaminlerin makarnadan suya

geçmesi şeklindeki kaybına ait olduklarına karar verildi. Bu vitaminlerin suda çözünen vitaminler olması, bu sonucu doğrulamaktadır. Makarna suyunun vitamin analizi, kaybın sadece suya geçme şeklinde olduğunu desteklemektedir.

Ayrıca, vitamin kayıp yüzdeleri hesaplandığında, 363 Kelvinde pişirme sırasında herbir vitaminin yüzde 40-50 den fazla bir kısmının suya geçtiği gözlenmiştir. Böylece makarna pişirme suyunun atılmaması gerektiği deneyler sonucu kesinleşmiştir.

**Anahtar kelimeler:** Yüksek Basınçlı Sıvı Kromatographisi, tiamin, niasin, riboflavin, vitamin kaybı, kinetic analiz, Arrhenius parametre.

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# CHAPTER 1

## INTRODUCTION

Vitamins are large, complex organic molecules which play important roles in human life. They are micronutrients since they are present in very small quantities in foods, compared to the amounts of other nutrients, such as proteins, carbohydrates and water; but yet they are very much necessary in order to have a completely nutritious diet. Due to their existence in negligible amounts, they can not be considered as energy sources in body.

Vitamins in general may be grouped into two classes depending on their solubility characteristics. The water soluble vitamins are thiamin (vitamin $B_1$), riboflavin (vitamin $B_2$), niacin (nicotinic acid and nicotinamide), vitamin $B_6$ group (pyridoxine, pyridoxal, pyridoxamine), panthothenic acid, folic acid (folacin), biotin, vitamin $B_{12}$ group (cobalamin) and vitamin C (ascorbic acid). The fat soluble vitamins, on the other hand, are vitamin A, vitamin D, vitamin E and vitamin K.

The roles and functions of vitamins in body are many. Vitamins are biologically active agents, like enzymes and hormones. Vitamins play a decisive role in the organism by regulating many metabolic reactions within the body.

They are controlling agents in many biological reactions in the organism, therefore vitamins are always needed for normal health, growth and good functioning of all organs. Most of the vitamins function as coenzymes, playing necessary roles in many enzymic reactions.

The human body can synthesize many of its enzymes and hormones, but not vitamins, especially in sufficient amounts. Thus, the supply of vitamins should be provided by the food. Many of the foods contain various vitamins and will provide the needed amounts of them, if they are used up in enough kinds and quantities. However, most of the vitamins are sensitive to heat, light, oxidation, pH changes and the presence of certain compounds. Therefore large amounts of vitamins are generally lost during the food preparation steps. Considerable losses of water soluble vitamins occur when the foods are cooked in water.

There are many studies for the preservation of vitamins in foods while they are processed. The knowledge on the amount of loss of vitamins helps to find ways to keep them in desired levels in foods during processing. Because, if the vitamins are not present in required amounts, some special diseases and complications may arise in living organism. For instance, the deficiency of thiamin results in beriberi, niacin deficiency causes pellegra and may generally lead to mental insta-

bility due to lack of ATP and riboflavin deficiency may result in ariboflavinosis and light sensitivity.

### 1.1 Aim of The Present Study

As mentioned in the first section, in order to have a healthy life, suitable amounts of vitamins should be included in family diet. For this, we must be able to know how much of these vitamins we are getting from foods depending on the processing conditions. It means that, we must know the kinetics of the losses of these vitamins during various processings, especially during cooking. Therefore, analysis of vitamins in cooked food samples gains a great importance.

The aim of this study was to find a good method for the combined analysis of thiamin, riboflavin and niacin in food systems, and to apply this method to a kinetic study of the losses of these vitamins.

As a food system, macaroni was chosen, since macaroni contains fairly large amounts of water soluble B vitamins and also it is one of the foods which is consumed in large amount in our country. Consumption of macaroni in recent years has increased in other countries too, because nutrionists suggest that the normal diet should contain more complex carbohydrates than the refined sugars, fats and cholestorel. Besides, the storage life of macaroni is longer than the other food products.

In our homes, macaroni is cooked in large amounts of water and this water is discarded after cooking. However, macaroni contains water soluble vitamins and most of them are also discarded with water. Another purpose of this study was then, to find the amounts of vitamins leaching into water during cooking at different temperatures and to suggest best cooking conditions for macaroni in home.

In this study the losses of thiamin, riboflavin and niacinamide in macaroni were studied. Kinetic studies involved measurement of concentration of substances against time. First, macaroni was cooked at different temperatures and the remaining amounts of vitamins in macaroni were analyzed with respect to time at each temperature. Therefore, the activation energies and frequency factors for the loss of thiamin, riboflavin and niacinamide were obtained during cooking of macaroni. However, the losses of these vitamins took place either by passage to the aqueous medium or by destruction, the calculated Arrhenius parameters included both of these factors.

Kinetics of vitamin losses in macaroni have been studied by Labuza [1] by using separate analysis methods for each vitamin. In this study, however a compact method for analysis was searched for three of these vitamins. The method of analysis is described in chapter 3.

## 1.2 Basic Information About The Vitamins Studied

### 1.2.1 Thiamin (Vitamin $B_1$)

Thiamin contains a pyrimidine moiety (2-methyl-4-amino-5-hydroxymethylpyrimidine) and a thiazole moiety (4-methyl-5-hydroxyethylthiazole) connected by a methylene group. Thiamin is found in organisms as thiamin monophosphate (TMP), thiamin pyrophosphate (TPP) and thiamin triphosphate (TTP) [2].

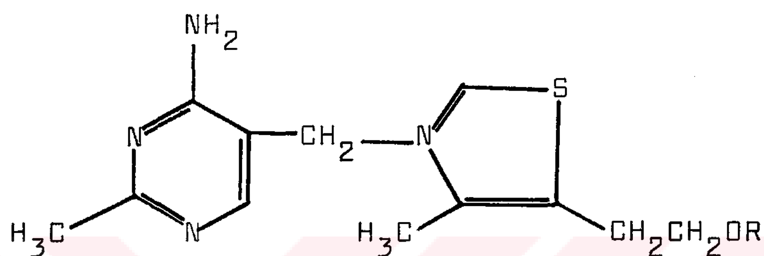
In foods, thiamin is present as in free form or in a combined form as a protein complex, as a phosphorus-protein complex, or as the phosphate esters. The relative amounts of any of these forms may vary in different types of products , but remain relatively constant in any one product [3].

Commercially thiamin is found as either thiamin hydrochloride or thiamin mononitrate. Thiamin hydrochloride is readily soluble in water (1 $g.ml^{-1}$), less soluble in methanol and glycerol and nearly insoluble in ether and benzene. It is most stable at acidic pH and it is heat labile. It melts with decomposition at about 521 K and absorbs moisture when exposed to air.

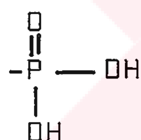
Thiamin mononitrate is a white crystalline powder having slight, characteristic odour. The compound melts with decomposition at 465-473 K and is non-hygroscopic. Thiamin mononitrate is less soluble in water than thiamin

hydrochloride (0.03 $g.ml^{-1}$), but in the solid state exhibits greater stability at temperatures lower than 368 K compared to hydrochloride form.

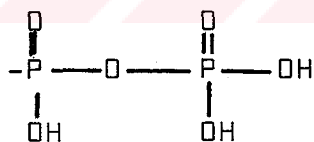
The structures of thiamin and its derivatives are shown below



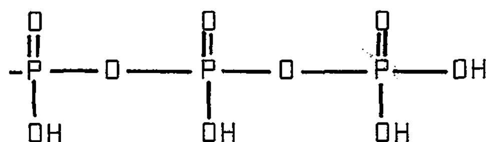

R : -H Thiamin



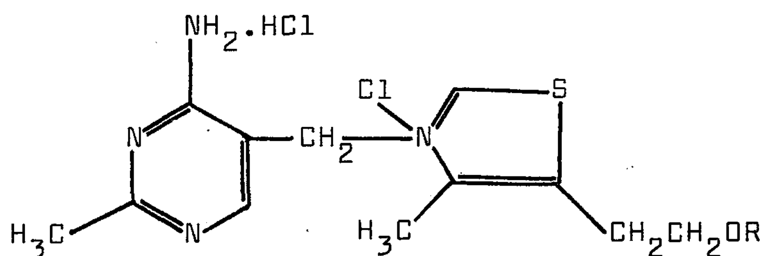

TMP




TPP

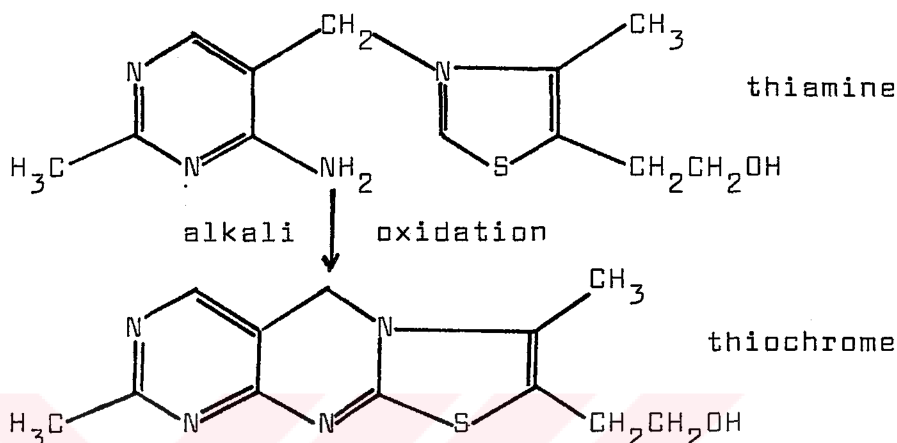



TTP




Thiaminchloride hydrochloride

Alkali oxidation of thiamin produces thiochrome which is the most important compound in some of the chemical determination methods of thiamin.




Recommended daily amounts of thiamin are 0.6-1.1 mg per day for children, 1.0-1.4 mg per day for adults.

Factors affecting availability of thiamin are cooking, some enzymes in food such as thiaminase, destruction by $CaCO_3$, nitrites, etc.. Some diseases also destroy thiamin (Gastrointestinal diseases) [4] .

Functions of thiamin can be summarized in a few sentences: It is a coenzyme in pyruvate metabolism, it plays important roles in growth, nerve activity, carbohydrate mechanism and energy production.

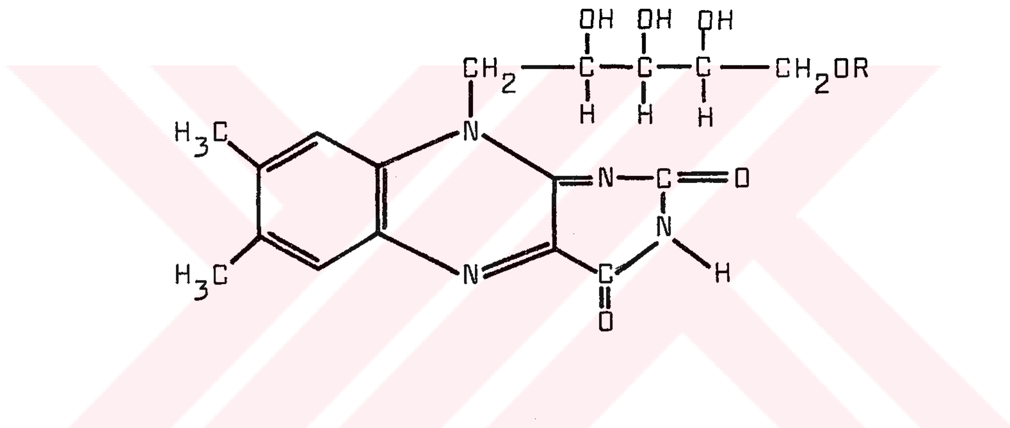
Generally all fruits, vegetables, nuts, grains and meats are the sources of thiamin. Most important sources of thiamin are wheat germ, rice bran, soybean flour, yeast and ham.

Thiamin deficency may lead to "beriberi" in humans.

This disease is characterized by rapid loss of weight, muscle wasting, marked peripheral, neuritis and muscular weakness. Deep reflexes are lost, sensory changes may occur, and anxiety states and mental confusion are evident [5] .

### 1.2.2 Riboflavin (Vitamin $B_2$)

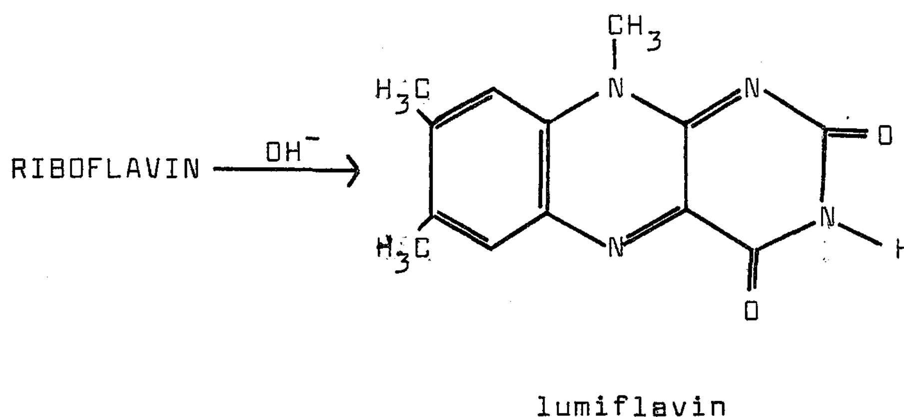
The structure of riboflavin is given below:



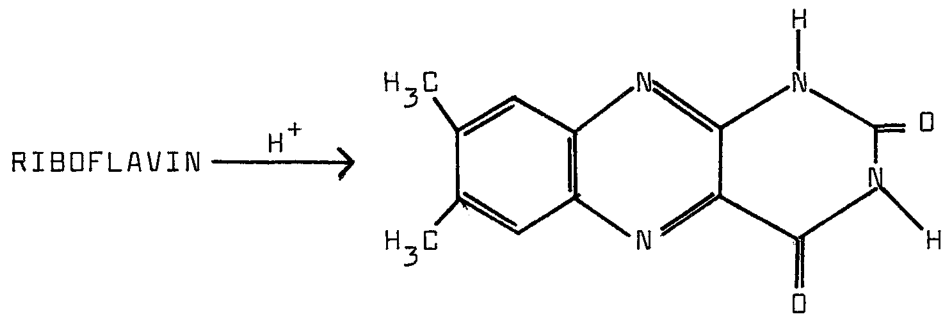

| R | Compound |
|---|---|
| R : -H | Riboflavin |
| : $-PO_3^{=}$ | FMN |
| : $-\overset{\displaystyle O}{\overset{\parallel}{P}}(O^-)-AMP$ | FAD |

Riboflavin, vitamin $B_2$ (7,8-dimethyl-10-(1'-D-ribityl) isoalloxazine) structurally is composed of an isoalloxazine ring with a ribityl side chain at the nitrogen at position 10. The major biological forms of the flavins are riboflavin and derivatives of it, namely, flavinmononucleotide (FMN) and flavin-adenine dinucleotide(FAD) [2].

Riboflavin is soluble in water and insoluble in acetone, chloroform, ether and benzene, it melts at about 553 K with decomposition. Generally, if foods are protected from light, increasing temperature does not increase its decomposition rate. Riboflavin in solution degrades rapidly when exposed to ultraviolet or visible radiation. For example, if milk is left under sunlight for 2 hours, more than 50 % of the riboflavin will be lost. Under acidic or neutral conditions, riboflavin looses the ribityl side chain to form lumichrome whereas in alkali solutions, riboflavin is photochemically converted to lumiflavin.




lumiflavin




lumichrome

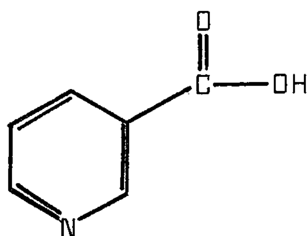
Recommended daily amounts are 0.6-1.2 mg per day for children, 1.5-1.7 mg per day for adults. There are some important factors which decrease the amount of riboflavin, such as cooking, exposure of foods to sunlight and some enzymes. Riboflavin acts as a coenzyme in respiratory enzyme systems, it is a constituent of flavoproteins and redox systems. It plays some important roles in maintenance of mucosal, eye tissues, growth and development of fetus.

Main sources of riboflavin are yeast, vegetables, fruits, nuts, and especially liver and kidney of beef, calf, chicken, pork and sheep.

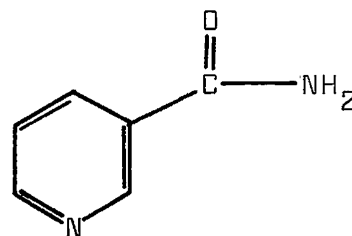
Riboflavin is not known to be the prime etiologic factor in a major human diseases, although patients with pellaga, beriberi and kwashiorkor are generally also deficient in riboflavin.

### 1.2.3 Niacin

Nicotinic acid and nicotinamide are known as niacin and niacinamide respectively. The structure of niacin and niacinamide are shown below:




Nicotinic acid




Nicotinamide

Both of them are active forms of this vitamin [6] . Niacin is unaffected by oxidizing agents, light or pH. It sublimes without decomposition. It is amphoteric and forms salts with acids and bases. A gram dissolves in 60 ml water. The melting point of niacin is 237 K. Nicotinamide has a melting point of 401-404 K. It can be recrystallized as needles from benzene. A gram of nicotinamide, dissolves in one ml water. The recommended daily amounts of niacin are about 9 mg for children and 18 mg for an adult. Taking oral antibiotics, cooking and some special diseases might affect the usage and availability of niacin. They function in metabolism as part of the coenzymes $NAD^+$ and $NADP^+$. They play some roles in hydrogen and electron transfer agents in carbohydrate metabolism. They furnish coenzymes for dehydrogenase systems. They are also coenzymes in lipid catabolism, oxidative

deamination and photosynthesis.

Niacin is widely distributed in plant and animal sources; including meat products, particularly liver, fruits, vegetables, nuts and microorganisms.

A common human nutritional disease caused by a deficiency of the vitamin and characterized by oral lesions diarrhea, dermatitis and neurological lesions is pellegra.

## 1.3 Methods For The Determination of Thiamin, Riboflavin and Niacinamide

There are several chemical, physicochemical and microbiological determination methods for water soluble vitamins. These methods are fluorometric, spectrophotometric, chromatographic (GC, HPLC), microbiological and analytical methods.

The selection of best method depends on the factors which are (i) type of data needed, (ii) number of samples and (iii) capabilities of laboratory.

### 1.3.1 Fluorometric Method

It is still a widely used technique for the determination of water soluble B vitamins, especially thiamin and riboflavin. The method is based on the measurement of the difference of fluorescence before and after treatment of vitamin with an oxidizing agent.

For the analysis of thiamin with fluorometer, thiamin must be converted to thiochrome, which fluoresces in

ultraviolet light, by oxidation in alkaline solution. In the absence of other fluorescingsubstances under standard experimental conditions, the fluorescence is proportional to the amount of thiochrome produced during oxidation and is a measure of thiamin present in an assay solution.

Lee and his co-workers [7] reported that after acidifying and enzymic treatment of the chicken sample, fluoremeter was used to analyze the thiamin content.

In the case of riboflavin, riboflavin gives a yellowish-green fluorescence in ultraviolet light which is characteristic of the compound and the intensity of the fluorescence is proportional to the concentration of the vitamin in the dilute solution. The maximum intensity is obtained between pH 6 and 7. The method involves the measurement of the difference of fluorescence before and after treatment with sodium hydrosulphite. After measuring the total fluorescence of the assay solution, riboflavin is reduced to non-fluorescent leuco-riboflavin by treatment with sodium hydrosulphite. The residual fluorescence of the solution is measured and the value thus obtained is subtracted from the main fluorescence reading. The difference in the two readings is used to calculate the amount of riboflavin present in the assay solution. The limitation of the method is that the treatment with sodium hydrosulphite does not always quench the fluorescence of vitamin $B_2$ alone. Since sodium hydrosulphite is

a powerful reducing agent, it also partially reduces the interfering substances in addition to vitamin $B_2$. For example thiochrome, which is the oxidation product of thiamin is particularly reduced by sodium hydrosulphite and its blue fluorescence is thus quenched.

This method is particularly reliable in the absence of other fluorescent meterials.

Ranhotra [8] , Kamman [1] and Dexter [9] used this technique for pasta products and semolina for both thiamin and riboflavin determination. Fluoremeter was used for the analysis of riboflavin in milk by Rashid [10] and for both vitamin in broccoli by Hudson [11] .

### 1.3.2 Gas Chromatographic Method

Gas chromatography is used for thiamin and nicotinic acid, although it is not advisable to use the method in the case of food system because of the low vitamin levels and large amounts of impurities in these materials.

This method involves the changing of the thiamin and nicotinic acid into a volatile derivative. For example, nicotinic acid is insoluble in organic solvents, has an acid group in its molecule and sublimes; hence it is not suitable for gas chromatographic analysis. Then it is determined as its ethyl ester or as N-ethylnicotinamide. A gas chromatography with a nitrogen-phosphorus detector has been used to quantitate thiamin in a number of meats, vegetables and cereals by Echols [12] . He found

that his result was in agreement with the results obtained by spectrophotometric analysis.

### 1.3.3 Microbiological Method

It is based on the observation that certain microorganisms require specific vitamins for their growth. In a basal medium complete in all respects, except for the vitamin under analysis, growth responses of the organism are quantitatively compared in the standard and in the assay solutions. The organisms must be specific for the vitamin and properties of the test strain must remain absolutely constant after prolonged subculture. Although they are extremely sensitive, microbiological assays are often not well suited for analysis because of their lengthyness and limited sample analysis capacity, poor precision and possible stimulation or growth inhibition by other compounds.

The amounts of thiamin and riboflavin were analyzed by Beetner [13] in cereals and by Augustin [14] in cooked potatoes with the microbiological methods.

Miyamato [15] reported a rapid microbiological assay for niacin. Incubating time is reduced from 18-24 to 3-4 hours by using heavy inoculation of exponentially growing Lactobasillus plantarum grown on a basal medium adjusted to pH 6 and containing 3 µg nicotinic acid and 50 µg vitamin free peptone per 50 ml. Bacteria responded molarly to nicotinic acid, nicotinamide and NAD for both

conventional and rapid assays, but only 40 % of the response is obtained for N-methylnicotinamide.

Johnson [16] also used microbiological assay methods for the determination of the vitamin composition of apples. He used Lactobacillus plantarum for niacin, Lactobacillus viridescens for thiamin and Lactobacillus casei for riboflavin.

### 1.3.4 Colorimetric Method

Nicotinic acid and its amide are treated with cyanogen bromide which breaks one carbon-nitrogen linkage, then it is allowed to react which can be determined colorimetri-metrically.

This method is not usable for the foods, because thiamin and pyridoxin interferes with the determination by giving the reaction with cyanogen bromide to give colored products, also. However, Ranhotro [8] used this technique for the niacin analysis of potatoes and Dexter [9] for niacin determination of spaghetti.

### 1.3.5 High Performance Liquid Chromatographic Method

High pressure liquid chromatography provides a simultaneous determination of different vitamins. It is time saving, accurate and most reliable method.

Wills and his co-workers [17] analyzed water soluble vitamins by using high pressure liquid chromatography on two bonded phase columns, μ Bondapak $C_{18}$ and μ Bondapak

$NH_2$. They determined isolated vitamins, $B_{12}$, pyridoxin, riboflavin, thiamin, niacin, niacinamide, folic acid and ascorbic acid by using the differences in their retention times. They studied various proportions of methanol and water added salts, such as the mobile solvent. They suggested that by proper selection of solvents, it was possible to analyze all eight vitamins with μBondapak $C_{18}$ and all except folic acid on Bondapak $NH_2$ with seven and six vitamins respectively, being separated in one analysis on each column.

The order of elution of vitamins from μBondapak $C_{18}$ was in the reverse order from μBondapak $NH_2$, so that vitamins that were not easily separated on one column were better separated on other.

Toma and Tabekhia [18] used a reversed phase $C_{18}$ column for the simultaneous quantitative analysis of niacin, thiamin and riboflavin in rice and rice products. Their mobile phase consisted of water-methanol-acetic acid (60:39:1, V/V) with the ion-pair modifiers pentone and heptane sulfonate in 0.005 $mol.lt^{-1}$ concentration. After simple acidification and filtration of sample, they measured the vitamins in a few minutes. The ultraviolet detector was set at 254 nm. The method was compared with the procedures of the AOAC method. The liquid chromatographic method proved to be rapid, accurate, and in good agreement with the official method.

There is another example for the analysis of vitamins which was reported by Kamman and Labuza [19] . They examined the determination of thiamin and riboflavin in cereals by both HPLC and fluorometric methods. As chromatographic condition, μBondapak $C_{18}$ was used at 254 nm, after preparing the sample by acidifying, with 0.1 N HCl, autoclaving, centrifuging and filtering.

They reported that HPLC method offers certain advantages including simultaneous analysis, wide equipment availability and rapid change over for alternative applications.

Ashoor [20] analyzed riboflavin in eggs and dairy products. He used μBondapak $C_{18}$ column and he reported that HPLC method was simple, sensitive and specific for riboflavin. The samples were eggs, whole milk, 2 % fat milk, skim milk, dry milk, yoghurt, cottage cheese, and cheddar cheese. Simple acidifacion and centrifugation was applied to all samples. The solvent was water-methanol-acetic acid (68:32:0.1 V/V) at a flow rate of $1 ml.min^{-1}$ and 270 nm was used for detection.

Niekerk [4] analyzed niacin, content of maize meal, high fiber biscuit, rice, spaghetti, mushrooms, soybean meat and breakfast cereal with the usage of HPLC by using a new technique, called automatic column switching. In this case, sample was subjected to chromatography on both reverse phase and anion exchange columns to ensure

adequate separation from interfering substances. Automatic column switching is used to transfer the niacin fraction from reverse phase to the anion-exchange column. In this report the result was compared with the result obtained from the microbiological techrique and reasonably good agreement was observed between them.

It is possible to analyze both water soluble and fat soluble vitamins together. Macrae [21] was able to analyze the complete range of isolated vitamins from the water soluble to the fat soluble in a single chromatographic run, especially using reverse phase packing and a wide polarity gradient. However he suggested that in the food applications, the extracts are contaminated with other compounds and the chromatographic procedure is not enough, but Bernd Glatz [22] had developed a method for the simultaneous determination of both water and fat soluble vitamins in food by using the same solvents with different gradients. He applied this technique to the determination of vitamins in chicken feed. The fat and water soluble vitamins are extracted and then analyzed as mentioned above.

Actually, there are many other reports dealing with the application of HPLC to the analysis of water soluble vitamins in foods [23-36] which will be too lengthy to be cited here.

## 1.4 Previous Studies On The Kinetics of Vitamin Losses In Food Systems

The nutritional implications of food processing and preventing possible losses of their nutritional value have become important in recent years.

To minimize the loss or destruction rates and their dependence on such factors as temperature and exposure time to that temperature must be determined.

The kinetic studies of these vitamins were performed in various foods. Mulley [3] studied the degradation kinetics of thiamin hydrochloride. Thiochrome fluorometric method was used for its determination in low acid foods. He prepared pea puree, beef puree, by necessary diluting and pasting, and prepared stock solution by dissolving thiamin hydrochloride in ethanol and this stock solution was buffered by adding phosphate buffer (pH 6). He heated these samples at 394, 399.6, 405.2, and 410.7 K upto 120 minutes and found that the activation energy for thiamin was 115.06 $kJ.mol^{-1}$ in peas, 114.64 $kJ.mol^{-1}$ in beef and 123.00 $kJ.mol^{-1}$ in phosphate buffer. Thermal destruction of thiamin hydrochloride in buffered solutions was found to be a first order reaction. However, deviations from first order reactions were observed in the food products.

The observation that thiamin in natural foods is more heat resistant than thiamin in aqueous and buffered solutions indicates the existence of factors, other than

heat, that can modify the reaction.

Proteins are known to protect thiamin even though the protective mechanism involved has not been elucidated Adsorption upon starch in foods may also play an important role in causing the retention of thiamin during heating. Variation in the proximate composition of foods does not seem to be of prime importance in accounting for differences in thiamin destruction rates. The presence of sodium chloride in solution also does not appear to affect the rate of thiamin breakdown to any great extent (3).

Kamman [1] reported the degradation of thiamin and riboflavin in enriched pasta as a function of temperature and water activity ($a_w$). He used Auto-Analyzer II system for measurement of thiamin and riboflavin. This method included a fluorometric determination of thiamin and riboflavin under continuous flow by a single extraction for both vitamins. Two systems, steady and unsteady conditions of 298, 308, 318 and 328 K, during the 80-320 day storage times, between five to seven sampling times were used. Unsteady conditions were the applications of fluctuating square wave temperature during storage. Water activity of the samples before packaging and at the end of storage study was measured by vapor pressure manometer. It was suggested that riboflavin, in the absence of light, was quite stable even at 328 K for 147 days.

But as the $a_w$ of the system is increased, the rate of loss of thiamin also increased. Kammen found an activation

energy of 128.87 kJ.mol$^{-1}$ for thiamin at an $a_w$ 0.44, 124.68 kJ.mol$^{-1}$ at an $a_w$ 0.54 and 112.30 kJ.mol$^{-1}$ at an $a_w$ 0.65. As a result, since water activity of pasta is generally between 0.44-0.50, riboflavin and thiamin losses should be insignificant, if pasta is stored at temperatures below 30$^0$C and relative humidity below 50 % for 12-18 months.

Woodcock [37] studied the effects of light, $a_w$ and temperature on the rate of riboflavin degradation in pasta. HPLC was used for the determination of riboflavin and lumichrome in enriched macaroni with fluorescence detector. Sample preparation was grinding the macaroni, acidifying with 0.1 N HCl, autoclaving, centrifugating for two times, then filtering. All analysis were carried out in subdued light. Reverse phase HPLC with a Bondapak $C_{18}$ column was used to separate and quantify riboflavin. Their mobile phase was 1 % glacial acetic acid, 43 % methanol and 56 % distilled water.

The fluorescence detector increases the sensitivity and specificity for determination of riboflavin and lumichrome at different wavelengths by taking into consideration their different fluorescence characteristics.

The stability of riboflavin and lumichrome was measured under steady state conditions of 298, 308 and 328 K. At each temperature, samples were stored over a salt solution at a water activity of 0.44. These samples

were then exposed to various light intensities.

Riboflavin in solution degradates rapidly when exposed to ultraviolet and visible radiation. Woodcock suggested that in order to better understand kinetics and mechanism of riboflavin losses, lumichrome production should also be measured. Under acidic or neutral conditions, riboflavin looses the ribityl side chain to form lumichrome whereas in alkali solution, riboflavin is photochemically converted to lumiflavin. These two flavins have not been found to have riboflavin activity. He showed that if the samples were protected from light, lumichrome was not produced.

This study showed that riboflavin degradation in pasta occurs in two distinct phases as lumichrome production occurs in two phases, too. Approximately 50 % of the initial riboflavin content was lost within 12 hours. This destruction follows first order kinetics and initial loss occurs rapidly accompanied by a rapid production of lumichrome.

At the second phase, a slower destruction rate for the riboflavin and a slower increase in production of lumichrome were found and this phase also was accepted as obeying to first order reaction kinetics. Woodcock calculated that the activation energy for the first phase range between 2.51 $kJ.mol^{-1}$ and 8.37 $kJ.mol^{-1}$ and for the second phase, it was ranged between 7.95 $kJ.mol^{-1}$

to 17.95 $kJ.mol^{-1}$.

There is not much information about the kinetics of the destruction of niacin or niacinamide in foods. However, Thompson [38] informed that the activation energy of niacin in asparagus was found to be 22 $kJ.mol^{-1}$ at the temperature range of 283 to 300 K.

# CHAPTER 2

# INTERPRETATION OF KINETIC DATA

The initial objective of experimental kinetic studies is the development of a mathematical model to describe the reaction rate as a function of experimental variables. The variables which affect the rate of a reaction, might be the temperature, time, pH or water activity and moisture [39] .

In general, the values of the rate constants and the activation energy are the most important parameters to be determined in kinetic studies. There are two methods for the determination of the rate constant from experimental data: One of them is the differential method and the other is the integral method [40] .

## 2.1 Differential Method of Determining Rate Constant of The Reaction

In experimental kinetic studies, it is impossible to measure the reaction rate itself. The measured quantity is the concentration of a reactant or product with changing time. The rate of a reaction can be defined as the change in the concentration of a reactant or product per unit time.

The rate of a reaction can be expressed as following with respect to the concentration of a reactant.

$$\text{Rate} = \upsilon = k(C_A)^n \qquad 2.1$$

where k is the rate constant, n is the order of the reaction and $C_A$ is the concentration of the reactant.

When the common logarithm of this equation is taken, we have

$$\ln \upsilon = \ln k + n \ln(C_A) \qquad 2.2$$

Therefore, if the rate is measured at various values of the reactant concentration, a double logarithmic plot of the rate against the concentration may give a straight line. The order of the reaction which is the dependence of rate on the concentration of the reactant, can be obtained from the slope of this straight line and the intercept on the $\ln \upsilon$ axis yields $\ln k$.

For the determination of the order of the reaction, two different procedures might be followed. These are the initial rate measurements and the differentiation of data from a single run.

In the initial rate measurements procedure initial rates are measured at various initial concentration. Tangents are drawn at the beginning of each reaction. The negative of the slopes of each of these represents the initial rate which corresponds to an initial concentration. Then, the logarithm of these initial rates ($\ln \upsilon_o$) are plotted with respect to the logarithm of the initial concentration ($\ln C_{Ao}$) according to equation 2.2

A schematic representation of this procedure is given below

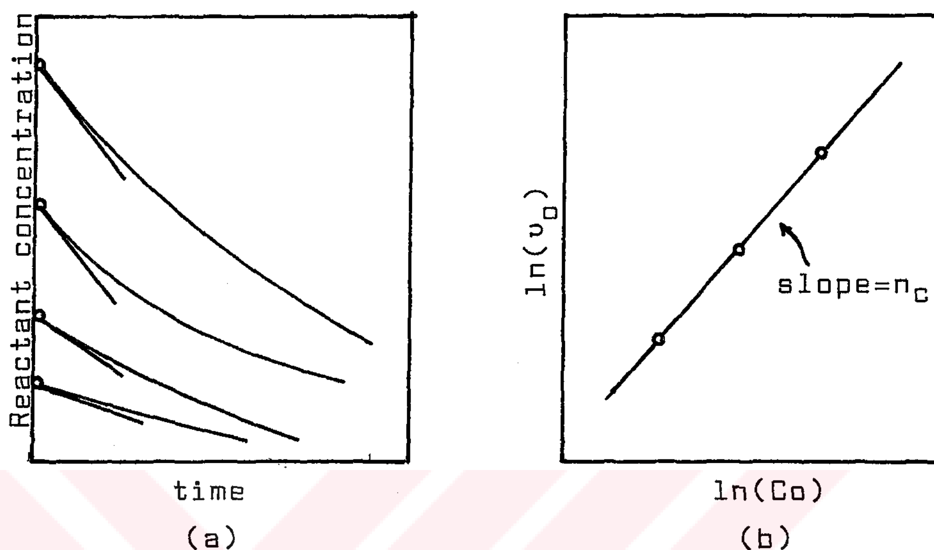



**Figure 1.** (a) The plot of concentration versus time. (b) The plot of the logarithms of the initial rates versus the logarithms of the corresponding initial concentrations.

The slope of the $\ln v_o$ $-\ln(C_{Ao})$ line gives the value of the order, which is now called the "true order of the reaction or order with respect to concentration ($n_c$)".

In the procedure of differentiation of data from a single run, one performs a single run starting with an initial concentration and the change in the concentration of a reactant is followed with respect to time. Then, by drawing tangents to the concentration-time curve at various reaction times, the rates are obtained corresponding to various reactant concentrations.

Later, logarithms of these rates are plotted as a function of the logarithms of respective concentrations and the slope yields the order of the reaction, which is now called "the order with respect to time ($n_t$)". The procedure is explained grahphically below

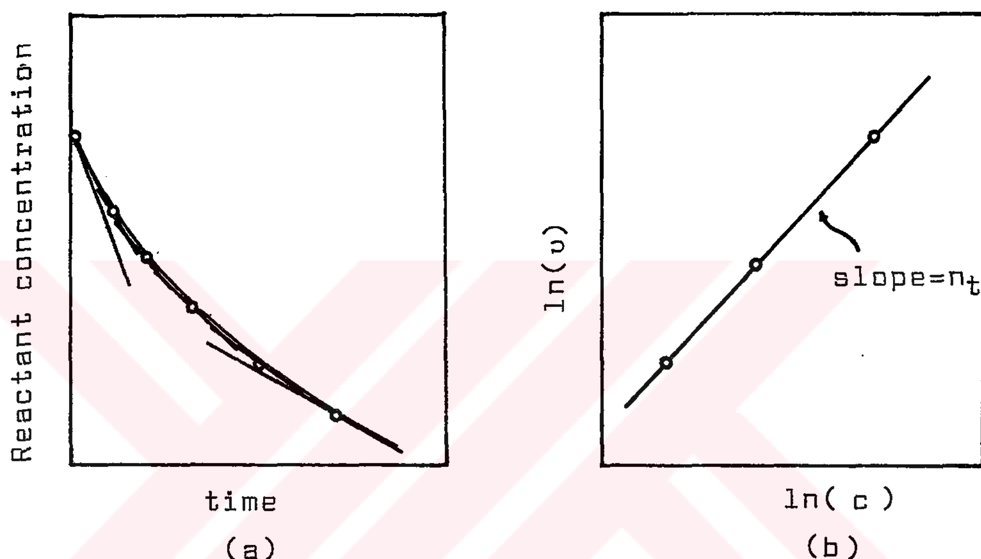
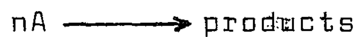



**Figure 2.** (a) The plot of concentration versus time. (b) The plot of the logarithms of the rates versus logarithms of the concentrations.

If there is no activator or inhibitor present in the reaction system, the values of the orders obtained from the above two procedures are expected to be the same. However, if there is an activator in the system, the order with respect to time is less than the true order. The presence of an inhibitor makes the true order smaller than the order with respect to time.

## 2.2 Integral Method of Determining Rate Constant of The Reaction

For a reaction of the type



$nA \longrightarrow$ products

The rate of disappearance of the reactant can be written as

$$\frac{-dC_A}{dt} = k(C_A)^n \qquad 2.3$$

After rearrangement, this equation can be written in the form

$$\frac{-dC_A}{(C_A)^n} = kdt \qquad 2.4$$

By giving different values to the order of the reaction n as 0, 1, 2 or 3, the above equation can be integrated between initial and final concentration of reactant A and between zero time and time t. The integrated rate expressions for the zero and first order reaction, thus obtained are:

$$C_{Ao} - C_A = kt \qquad n=0 \qquad 2.5$$

$$\ln \frac{C_{Ao}}{C_A} = kt \qquad n=1 \qquad 2.6$$

When using this integral method in kinetic analysis, one uses the integrated rate expressions directly and

searches for agreement with the experimental data. Thus, if the order is assumed to be equal to one, equation 2.6 is used and measured $C_A$ values are plotted as $-\ln(C_A)$ versus time. If a straight line is obtained, this tells the agreement of the experimental data with the integrated first order rate expression and the slope of the straight line gives the value of the rate constant. If, instead of a straight line, a deviation from the linearity is observed, this tells that the assumed order is not suitable for the reaction. Then another order is assumed and the integrated rate expression for that order is tried for the experimental values as explained before.

The next step in kinetic studies is the determination of the activation energy for the reaction. The relation between the experimental rate constant and the activation energy of the reaction is expressed by the Arrhenius equation.

$$k = Ae^{-E_a/RT} \qquad 2.7$$

where A is a constant and independent of temperature, known as frequency factor, $E_a$ is the activation energy, R is the gas constant ($8.314 J.K^{-1} mol^{-1}$) and T is the absolute temperature.

The activation energy for a reaction, which is defined as the amount of necessary energy that the

reactants should acquire to surmount the potential energy barrier in order to form the product, can be obtained by measuring the rate constants of the reaction at different temperatures. Thus, lnk values are plotted with respect to the reciprocal of temperatures in Kelvins and the slope yields the experimental activation energy.

$$\ln k = \ln A - \frac{E_a}{R} \cdot \frac{1}{T} \qquad 2.8$$

From the intercept of the straight line on lnk axis; when 1/T equals to zero, ln of the frequency factor A is obtained.

The most common method to estimate the Arrhenius parameters (frequency factor and the activation energy) is the usage of "two-step linear least squares" method. In this method, the first regression is used to derive the rate constant which is then regressed versus 1/ absolute temperature to obtain the estimates for $E_a$ and A.

On the other hand, some scientists prefer using another method called the "nonlinear least squares". In this method, one step procedure is applied to obtain the frequency factor and the activation energy of the reaction from the whole experimental data.

For a zero order and first order reaction; the integrated rate expressions are

$$C_A = C_{A_0} + kt \qquad 2.9$$

$$C_A = C_{Ao}\exp(-kt) \qquad 2.10$$

These equations may be combined with Arrhenius equation (2.7) and the following expressions may thus be obtained.

$$C_A = C_{Ao} + At\exp(-E_a/RT) \quad \text{for a zero order reaction} \qquad 2.11$$

$$C_A = C_{Ao}\exp(-At\exp(-E_a/RT)) \quad \text{for a first order reaction} \qquad 2.12$$

For the deterioration of a single reactant following a zero or a first order model, following equations were drived by Cohen [41] .

For a zero order reaction

$$\ln(C_A) = \ln(C_{Ao}) + \ln(1+t\exp(\alpha-(E_a/R)(1/T-\beta))) \qquad 2.13$$

For a first order reaction

$$\ln(C_A) = \ln(C_{Ao}) - t\exp(\delta-(E_a/R)(1/T-\beta)) \qquad 2.14$$

where

$\alpha = \ln( A/C_{Ao}) - \beta(E_a/R)$

$\beta = (\Sigma(1/T)\omega_i)/(\Sigma\, \omega_i) \qquad i=1, ., ., .,n$

$\delta = \ln A - \beta(E_a/R)$

n = number of data points

$\omega$ = weighting factor

By using these equations and by applying some necessary statistical forms the nonlinear least squares

may be used for the determination of $E_a$, A and $C_{Ao}$ without calculating the rate constant.

This method decreases the errors of the calculated Arrhenius parameter to a great extent, but it requires specific computer programs for its application.

# CHAPTER 3

# EXPERIMENTAL

## 3.1 Selection of The Experimental Analysis Technique

One of the aims of this study was to choose a good method for the simultaneous determination of thiamine, riboflavin and niacinamide in macaroni samples. The examination of the literature data indicated that HPLC was the superior method among the other measurement techniques. There are many reports concerning the advantages of using HPLC for vitamin analysis. Some of these advantages can be summarized as follows:

1. HPLC is easy to automate, and it is especially useful for routine works.
2. HPLC has the capability of analyzing more than one sample at a time thus saves the equipment.
3. HPLC method has the ability of separating the analyzed compound from interfering substances.
4. HPLC method is rapid, sensitive and accurate.

Because of all the reasons mentioned above, in this study, HPLC method was selected for the analysis of vitamins.

## 3.2 High Pressure Liquid Chromatography (HPLC)

Chromatography is a separation method based on the differential migration of solutes through a system of two phases, one of which is mobile. There are many kinds of chromatography, but chromatographic methods generally fall into two main groups, gas chromatography and high pressure liquid chromatography.

HPLC is accomplished by injection of a small amount of liquid sample into a moving stream of liquid known as the mobile phase that passes through the column packed with particles of a stationary phase. As in the gas chromatography, separation of a mixture into its component depends on different degrees of retentions of each component in the column. The extent to which a component in the column is separated will be determined by its partitioning between the liquid mobile phase and the stationary phases. A variety of HPLC separation techniques that utilize different stationary and mobile phases have been developed [42,43] .

The basic HPLC chromatography consists of a solvent supply, a pump, an injection system, a column and a detector coupled to a chart recorder as shown in figure 3.

The solvent reservoir may be a convenient vessel, but provision must be made for degassing the solvent,

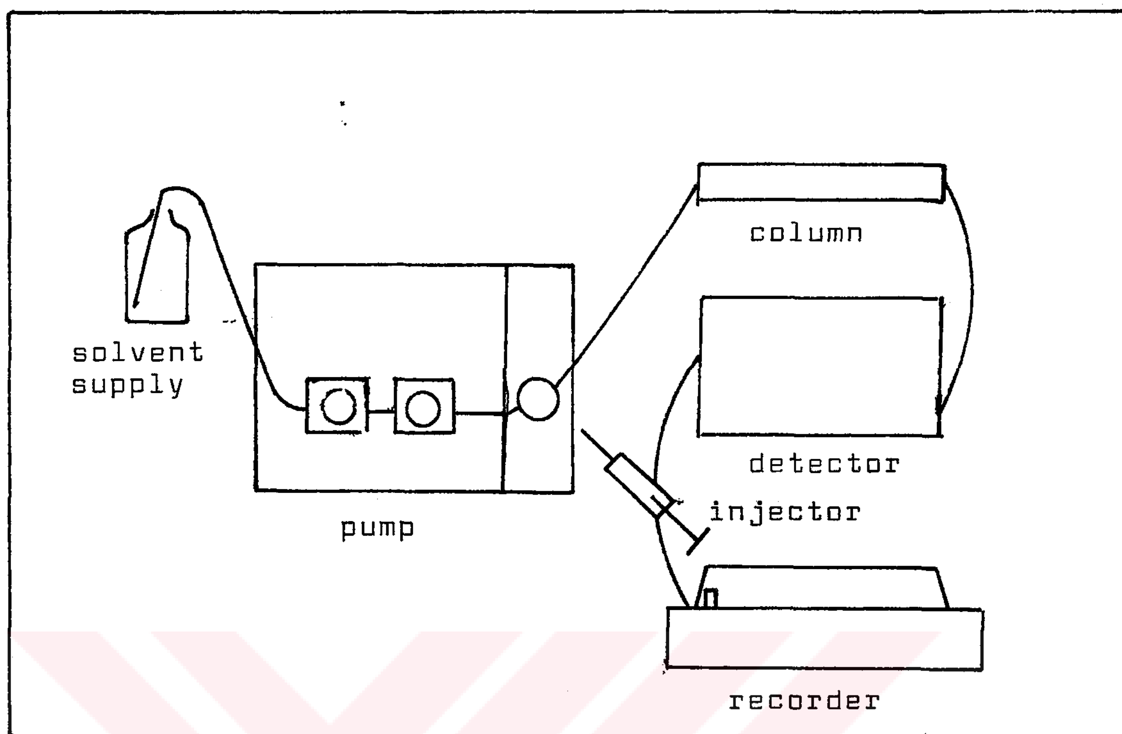



**Figure 3.** The components of HPLC

either by application of vacuum or heat or by ultra-sonification. The best thing is to use a solvent clarification kit to degas and filter the solvent. Failure to degas, particularly protic solvents may lead to air bubble in the detection cell, with consequent disruption of the chromatogram. Solvents are changed according to the kind of column used. The solvent selection is depended on the polarity of the column and the properties of the sample.

Chromatographic pumps are of two main types, constant pressure or constant displacement. Usually the former is air driven and pneumatically amplified and the latter

reciprocating and electrically driven. Generally the pump is a twin-headed pump. The flow produced by this type of pump is governed by the speed of the electric motor, which is readily controlled electronically to provide a gradient maximum. Operating pressures are in the order of 100 to 6000 psig, and this pump provides a maximum flow of 9.9 $ml.min^{-1}$.

Sample injection into the column may be carried out by one of two main methods, "on column" or via a valve. The former mode, septum or septumless, allows the sample discharged from the syringe directly into the top of the column. Injection valves, in which the sample is introduced into a holding-loop prior to application to the column, are more convenient in operation.

Analytical HPLC columns are usually constructed from stainless steel with zero-dead-volume reducing unions at the ends. The lengths of the columns ranged between 200-500 mm, most commonly 250 mm, with internal diameters of 3-5 mm.

The wide range of chemical compounds that are analyzed as food components in HPLC means that a wide range of differing column packing materials come into consideration. The most commonly used stationary phase is silica, which may be used unmodified, where the chromatographic process is one of adsorption, or a silica modified by bonding functional groups to the silanol groups to give a

partition system. Silica can be used with a wide range of solvents covering a large polarity range, although very polar compounds, such as aminoacids or sugars are more conveniently chromatographed on modified silica phases. When the group bonded to silica is hydrophobic in nature, for example a $C_{18}$ group, the stationary phase operates in reverse mode, where the less polar (more hydrophobic) compounds are more strongly retained [21].

The most commonly used columns in HPLC are μBondapak CN, μBondapak $NH_2$, porasil groups and μBondapak $C_{18}$.

The large range of detectors are currently available for use with HPLC systems, but of these only the ultraviolet detector, the fluorescence detector and the differential refractometer are in common use. Ultraviolet detectors can operate at a fixed wavelength, usually 254 nm with a mercury lamp, or over the whole ultraviolet range, using a deuterium lamp. The fixed wavelength detector has a higher sensitivity, because of the greater intensity of incident radiation, but its applicability is limited. The variable wavelength detector can be adjusted to the wavelength of maximum absorption of the compounds of interest and in addition, some variable wavelength detectors have a tungsten source which extends detection to the visible region.

The output from the detector is fed to a chart recorder or possibly, to a data handling integration

system, where routine analysis are being carried out.

The goal of a chromatographic separation is to match the sample of interest to the most appropriate column and again to match the solvent to the kind of column, such as polarity index of packing materials.

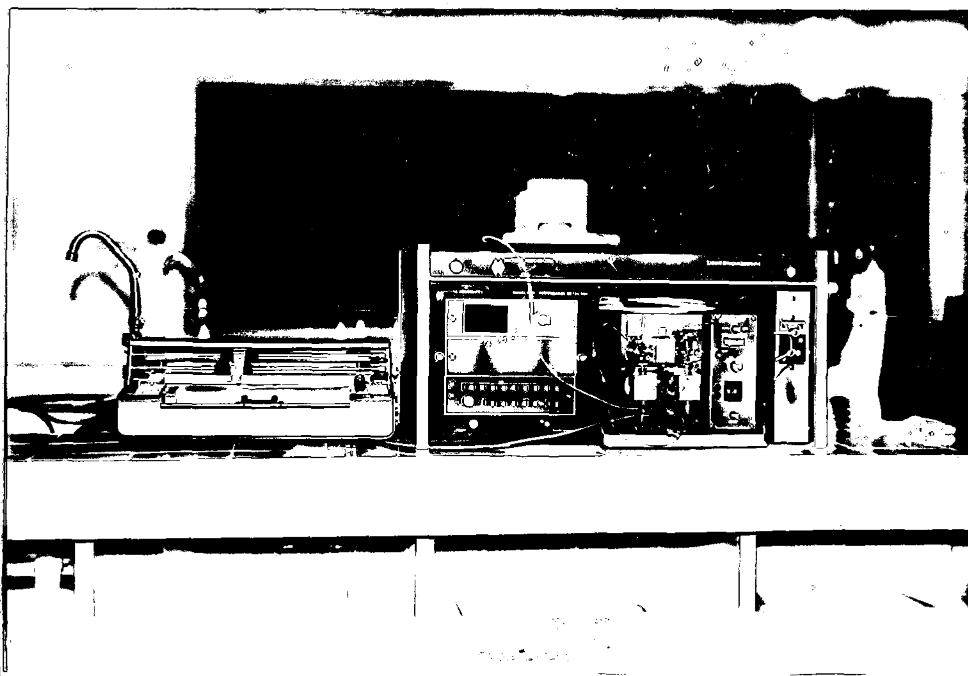
## 3.3 Description of The Analytical Part

### 3.3.1 Instrumentation of HPLC

The HPLC (Waters Associates, Inc.) system used in this study, contains a dual beam UV-visible absorbance detector referred to as Model 440, operates at a fixed wavelength of 254 nm with a mercury lamp.

The pump of this system was dual piston reciprocating pump referred to Model 6000A solvent delivery system. As an injection valve, Model U6K injector was used. The recorder of this system was omniscribe recorder.

A photography of HPLC used is given below.



### 3.3.2 Column Selection

For the analysis of water soluble vitamins in foods, various columns are used by different workers, including μBondapak $C_{18}$ and μBondapak $NH_2$, although they have different polarities. However, μBondapak $C_{18}$ found more frequent uses for this purpose.

In this study, μBondapak $NH_2$ was chosen as the column for HPLC since it was already present in our laboratory. μBondapak $NH_2$ is an intermediate polarity packing, consisting of a monomolecular layer of amino propylsilane for normal phase or weak anion-exchange chromatography. Dimension of this column is 3.90 mm in internal diameter and 30.00 cm column length.

### 3.3.3 Solvent Selection and HPLC Condition

Separations by liquid chromatography depend on both the stationary phase and the mobile phase, which are the column and the solvent respectively.

For the analysis of water soluble vitamins, generally water and methanol pair was used as the mobile solvent by many workers. The reason for this choice comes from the solubility of these vitamins in water. Since μBondapak $NH_2$ is intermediate in polarity, $H_2O$ is a highly polar solvent, methanol was added to water in order to bring the properties of mobile solvent and column close to each other.

Several preliminary studies were performed to find the best composition of the mobile solvent. In these experiments, each vitamin solution was injected separately to determine the individual retention times (the retention times can be explained as the time required after sample injection for the solute peak to appear at the end of the chromatographic column) and the macaroni sample was injected to analyze the degree of resolution. These solvents were filtered and degassed by using vacuum pump or using ultrasonic bath prior to use.

Studies to find the best components of the solvent were discussed below.

a. Water-methanol-hydrochloric acid combination:
Various combinations were studied such as the volume ratios methanol:water, 20:80, 30:70, and 50:50. In all these combinations, pH was adjusted to 3-4 by the addition of hydrochloric acid.

b. Water-methanol-citric acid combination:
Citric acid was used to adjust the pH of the methanol-water (20:80) mobile solvent in various amounts instead of hydrochloric acid. 0.1, 0.125 and 0.4 % citric acid were added to mobile solvent. Unfortunately, the results showed that the resolution of the vitamins in the macaroni samples was not good enough to use this combination.

c. Water-methanol-sodium citrate combination:
Sodium citrate was added to examine the effect of salt on the resolution of vitamins. 0.075, 0.25, 0.5 and 1.0 percent sodium citrate were added to methanol-water (20:80) after adjusting the pH of

this solvent to 3-4.

The effect of sodium citrate addition on the retention times of these vitamins are given in table 1.

**Table 1. Retention Times of Thiamin, Riboflavin and Niacinamide In Various Compositions of Sodium Citrate**

| Sodium | Retention Times (cm) | | |
|---|---|---|---|
| Citrate(%) | Thiamin | Riboflavin | Niacinamide |
| 0.075 | 0.70 | 1.40 | 1.30 |
| 0.250 | 0.70 | 1.30 | 1.32 |
| 0.500 | 0.70 | 1.40 | 1.35 |
| 1.000 | 1.40 | 1.00 | 1.15 |

As seen from the above data, the effects of sodium citrate in concentrations between 0.075-0.500 % are almost the same for all vitamins. The addition of 1 % sodium citrate, on the other hand increased the retention times of thiamin, but decreased those of riboflavin and niacinamide. This indicates that the low percentages of sodium citrate would give better separation as can be seen. However, the separation was not sufficient enough for the resolution of all vitamins.

First two trials were applied to the macaroni samples not to the standard solutions of the vitamins, in order to analyze the separability of the peaks of chromatogram. The increase of methanol percentage didn't increase the separability, so 20 percent of methanol was enough for the determination of vitamins.

After taking into consideration of these studies, it was decided that the methanol:water (20:80) using hydrochloric acid for pH adjustment was the best solvent system to analyze thiamin, riboflavin and niacinamide in macaroni.

By this solvent composition with flow rate of 1.3 ml per min, the following retention times were obtained for three vitamins.

**Table 2. Retention Times of Thiamin, Riboflavin and Niacinamide**

| Vitamin | Retention Times (cm) |
|---|---|
| Thiamin | 1.3 |
| Riboflavin | 2.5 |
| Niacinamide | 1.7 |

The sensitivity of Model 440 was 0.1 AUFS (absorbance unit fullscale). The chart speed of recorder was 10/10 $cm.min^{-1}$.

### 3.4 Cooking of Macaroni and Sample Preparation

All the macaroni used in this study was taken in 1986 from market. Percent moisture in this macaroni was analyzed to be 12.33.

Cooking temperatures of the macaroni were 323, 348, 353, 358 and 363 K. The cooking procedure can be outlined as following.

First of all a water bath was heated to the temperature required. Then 100 ml of distilled water was put into each of four 250 ml balloons and waited until the water inside the ballons come into equilibrium with the temperature of water bath. 5 g. of macaroni were introduced to each of these balloons and were kept there for various times. The cooking times were determined to be 10, 20, 30 and 40 minutes. Thus, with 10 minutes intervals, one of the balloons were taken from the water bath. The macaroni inside the balloons was quickly filtered and placed in the refrigerator at 273 K for one night. It is then put in a desiccator and vacuum dried until it becomes to a constant weight. This dried macaroni was ground finely by using an electrical grinder. Four grams of this ground macaroni was dissolved in 50 ml of 0.1 N HCl.

The solution was autoclaved for 30 min at 15 psi. The decanted suparnatant was filtered by blue-band filter paper after cooling to room temperature. Filtered

supernatant was passed through the sample clarification kit to remove undesirable contaminants and to increase the column life. The sample clarification kit includes a $10^{-2}$ml glass syringe, a number 17 cannula, a smooth-tip forceps, two swinny-filter holders and (Millipore) filters and prefilters.

The schematic representation of clarification kit is shown below.

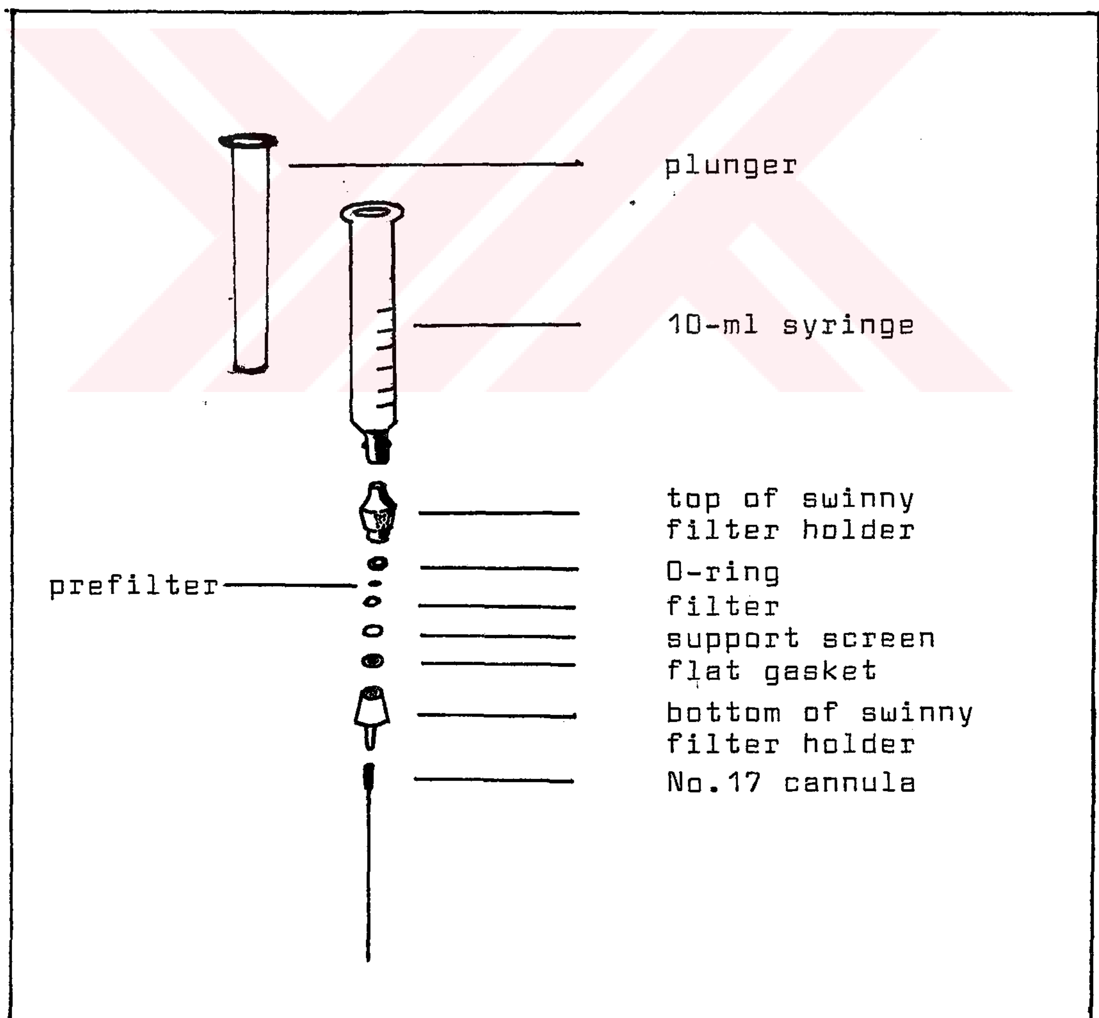



**Figure 4.** The sample clarification kit.

The samples were either analyzed immediately by injection $10^{-2}$ml of solution to the HPLC or were kept at 277 K in refrigerator for a night and then injected to chromatography. All of the samples were protected from sunlight at all steps in these procedures.

### 3.5 A Premilinary Study On The Determination of Vitamins In Enriched Macaroni

Since 1986, the macaroni products were enriched by the addition of thiamin, niacinamide, riboflavin and iron in one of the macaroni factories in Gaziantep. Both enriched and unenriched macaroni samples were prepared as described in section 3.4 without applying cooking procedure and by dissolving 5 g. macaroni in 30 ml 0.1 N HCl. The HPLC condition was different from the analysis which was explained in section 3.3, although the mobile phase was same, methanol-water (20:80, pH 4). The flow rate of mobile solvent was 2.3 $ml.min^{-1}$, sensitivity of absorbance was 0.5 AUFS and the chart speed of recorder was 5 cm/10 min. At this condition, the calibration curves were obtained also. After obtaining their chromatograms, the peak heights were measured and then concentrations of vitamins in both unenriched and enriched macaroni were calculated by using calibration curves.

The results were listed in Table 3. The calculated percent values of vitamins indicate that there is not

much difference in the vitamin content. However, these results might carry some uncertainties because they were preliminary analysis with different HPLC conditions compared to the finally selected procedures.

**Table 3. Comparison of The Vitamin Contents of Enriched and Unenriched Macaroni**

| Vitamin | | Peak heights (cm) | Concentration ($mg.ml^{-1}$) | % Vitamin content |
|---|---|---|---|---|
| Thiamin | Unenriched | 3.9 | 0.030 | 0.018 |
| | Enriched | 5.0 | 0.047 | 0.028 |
| Riboflavin | Unenriched | 3.5 | 0.045 | 0.027 |
| | Enriched | 4.4 | 0.060 | 0.036 |
| Niacinamide | Unenriched | 0.8 | 0.020 | 0.012 |
| | Enriched | 1.0 | 0.025 | 0.015 |

# CHAPTER 4

# EXPERIMENTAL RESULTS AND TREATMENT OF DATA

In this chapter, results of experimental studies and the treatment of these data will be given. This procedure will be followed for each of the vitamins analyzed, thiamin, niacinamide and riboflavin.

## 4.1 Calibration Curves

35 mg of each of the vitamins was dissolved in 500 ml of distilled water, corresponding to 0.07 mg vitamin per ml of water. Using this stock solution, different samples were prepared in various concentrations by necessary dilutions. After the injection of $10^{-2}$ml of each of the standard solutions, the peak heights were measured from HPLC chromatogram.

The concentration and the peak height data for each of the vitamins analyzed were shown in the following table (table 4).

The calibration curves were all presented in figures 5, 6 and 7 for thiamin, niacinamide and riboflavin, respectively.

**Table 4. Calibration Data for Thiamin, Niacinamide and Riboflavin**

| Vitamin | Concentration ($mg.ml^{-1}$) | Peak height (cm) | Retention time (cm) |
|---|---|---|---|
| | 0.0100 | 3.50 | |
| | 0.0125 | 4.60 | |
| Thiamin | 0.0200 | 7.50 | 1.30 |
| | 0.0250 | 9.87 | |
| | 0.0300 | 11.80 | |
| | 0.0100 | 2.40 | |
| | 0.0125 | 3.75 | |
| Niacinamide | 0.0250 | 7.00 | 1.70 |
| | 0.0500 | 13.20 | |
| | 0.0650 | 18.00 | |
| | 0.0200 | 5.77 | |
| | 0.0400 | 10.50 | |
| Riboflavin | 0.0500 | 13.20 | 2.50 |
| | 0.0600 | 16.10 | |

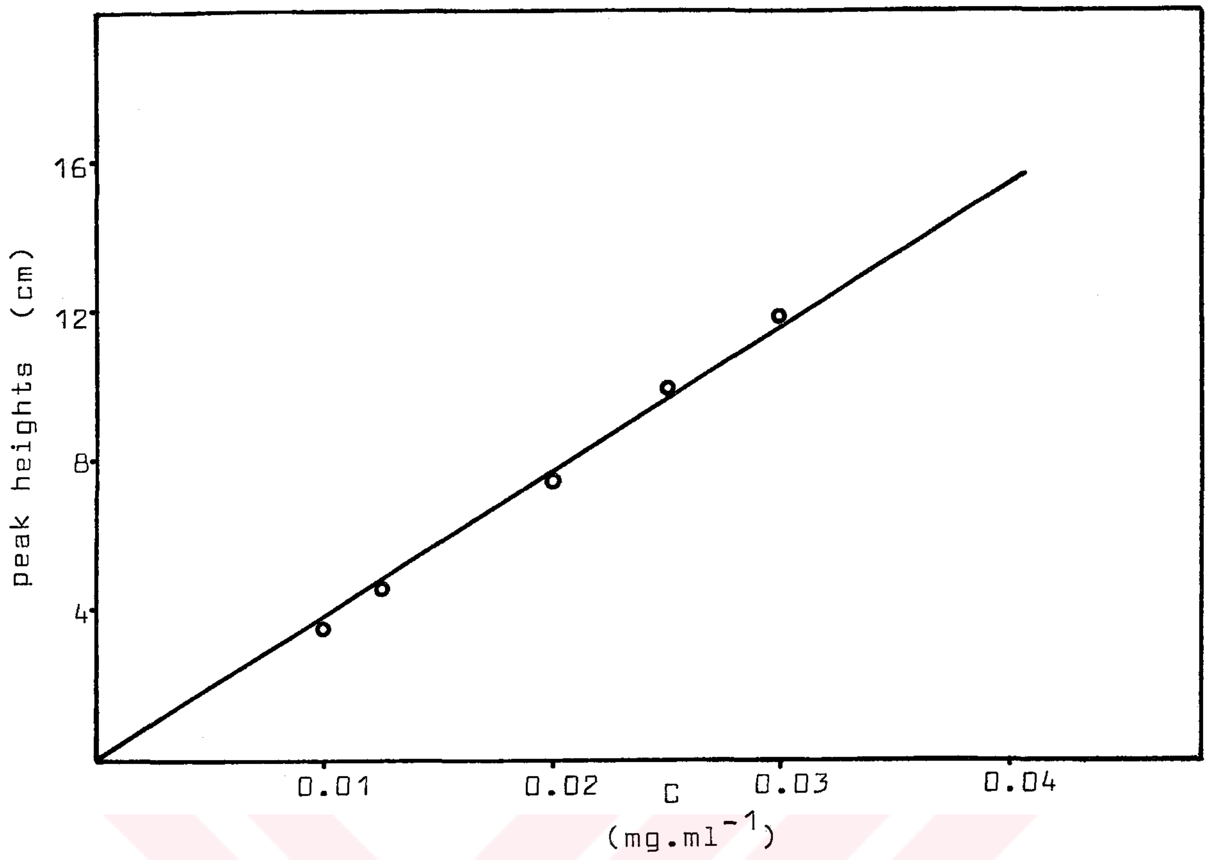



Figure 5. The calibration curve of thiamin.

20
15
10
5
peak heights (cm)
0.02
0.04
0.06
C
(mg.ml$^{-1}$)

Figure 6. The calibration curve of niacinamide.

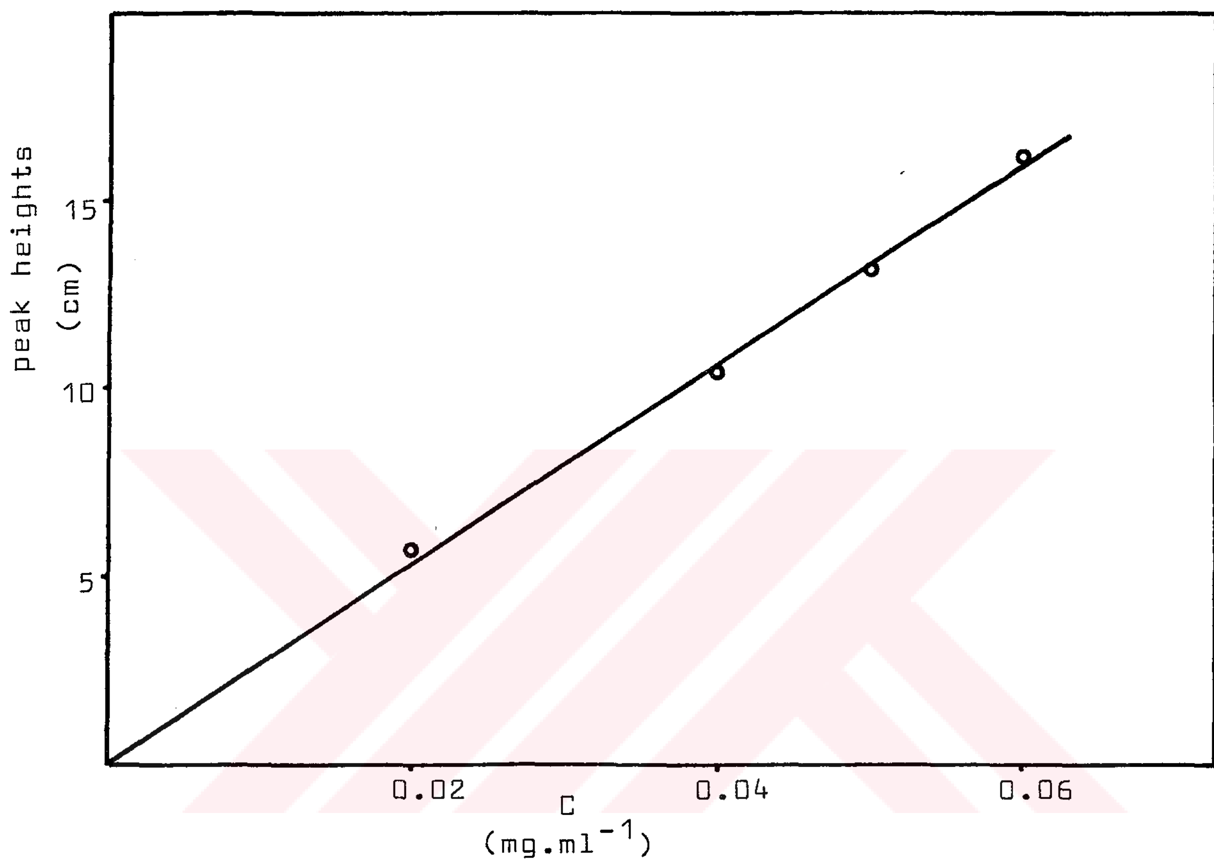



**Figure 7.** Calibration curve for riboflavin.

## 4.2 Results of Cooking Experiments

The macaroni was cooked at five different temperatures and samples were prepared for analysis as explained in section 3.4. After obtaining peak heights, the concentrations of the vitamins were measured by using their calibration curves. The peak heights obtained are the average of at least two injection. The peak heights and measured concentrations were listed at each temperature in tables 5, 6 and 7 for thiamin, niacinamide and riboflavin, respectively.

## 4.3 Treatment of Data

After cooking the macaroni samples for different time intervals at five different temperatures, the concentrations of retained vitamin were measured by using HPLC analysis technique. The logarithms of retention of concentration of vitamins versus time were regressed to calculate the rate constant. In these regressions, the extreme values of $C_A$ were omitted. $-\ln(C_A)$ versus time(min) graphs of thiamin at 323, 348, 353, 358 and 363 K were represented in the figures 8 to 12; the graphs of niacinamide, at 323, 348, 353 and 363 K, in figures 13 to 16; and the graphs of riboflavin at 323, 348, 353 and 363 K, in figures 17 to 20.

Then by using two step linear least squares technique Arrhenius parameters were obtained. ln of rate

Table 5.Concentration and Time Data for Thiamin

| T (K) | time (min) | peak height (cm) | concentration ($mg.ml^{-1}$) |
|---|---|---|---|
| 323 | 10 | 10.00 | 0.0260 |
| | 20 | 9.40 | 0.0245 |
| | 30 | 10.90 | 0.0285 |
| | 40 | 8.20 | 0.0210 |
| 348 | 10 | 6.60 | 0.0170 |
| | 20 | 4.20 | 0.0107 |
| | 30 | 5.10 | 0.0130 |
| | 40 | 4.30 | 0.0110 |
| | 50 | 4.40 | 0.0115 |
| 353 | 10 | 8.85 | 0.0230 |
| | 20 | 9.40 | 0.0245 |
| | 30 | 6.60 | 0.0170 |
| | 40 | 6.75 | 0.0175 |
| 358 | 10 | 6.60 | 0.0170 |
| | 20 | 4.70 | 0.0123 |
| | 30 | 4.40 | 0.0115 |
| | 40 | 5.20 | 0.0135 |
| 363 | 10 | 11.97 | 0.0310 |
| | 20 | 10.00 | 0.0260 |
| | 30 | 9.20 | 0.0240 |
| | 40 | 6.60 | 0.0170 |

Table 6. Concentration and Time Data for Niacinamide

| T (K) | time (min) | peak height (cm) | concentration ($mg.ml^{-1}$) |
|---|---|---|---|
| 323 | 10 | 5.50 | 0.0198 |
| | 20 | 5.15 | 0.0183 |
| | 30 | 5.30 | 0.0190 |
| | 40 | 4.15 | 0.0148 |
| 348 | 10 | 3.50 | 0.0125 |
| | 20 | 2.50 | 0.0095 |
| | 30 | 2.40 | 0.0085 |
| | 40 | 2.00 | 0.0070 |
| | 50 | 2.00 | 0.0070 |
| 353 | 10 | 4.60 | 0.0160 |
| | 20 | 3.85 | 0.0138 |
| | 30 | 3.05 | 0.0110 |
| | 40 | 3.50 | 0.0125 |
| 358 | 10 | 4.85 | 0.0173 |
| | 20 | 3.60 | 0.0130 |
| | 30 | 3.90 | 0.0140 |
| | 40 | 3.60 | 0.0130 |
| 363 | 10 | 4.45 | 0.0162 |
| | 20 | 3.40 | 0.0120 |
| | 30 | 3.30 | 0.0115 |
| | 40 | 1.95 | 0.0068 |

Table 7. Concentration and Time Data for Riboflavin

| T (K) | time (min) | peak height (cm) | concentration ($mg.ml^{-1}$) |
|---|---|---|---|
| 323 | 10 | 4.66 | 0.0165 |
| | 20 | 4.25 | 0.0150 |
| | 30 | 3.10 | 0.0105 |
| | 40 | 2.70 | 0.0095 |
| 348 | 10 | 3.97 | 0.0145 |
| | 20 | 2.40 | 0.0085 |
| | 30 | 2.00 | 0.0070 |
| | 40 | 1.40 | 0.0050 |
| | 50 | 1.20 | 0.0045 |
| 353 | 10 | 3.10 | 0.0105 |
| | 20 | 2.40 | 0.0090 |
| | 30 | 1.60 | 0.0060 |
| | 40 | 1.60 | 0.0060 |
| 358 | 10 | 3.35 | 0.0124 |
| | 20 | 2.30 | 0.0083 |
| | 30 | 2.05 | 0.0076 |
| | 40 | 1.80 | 0.0065 |
| 363 | 10 | 3.70 | 0.0138 |
| | 20 | 2.10 | 0.0080 |
| | 30 | 2.00 | 0.0075 |
| | 40 | 1.48 | 0.0053 |

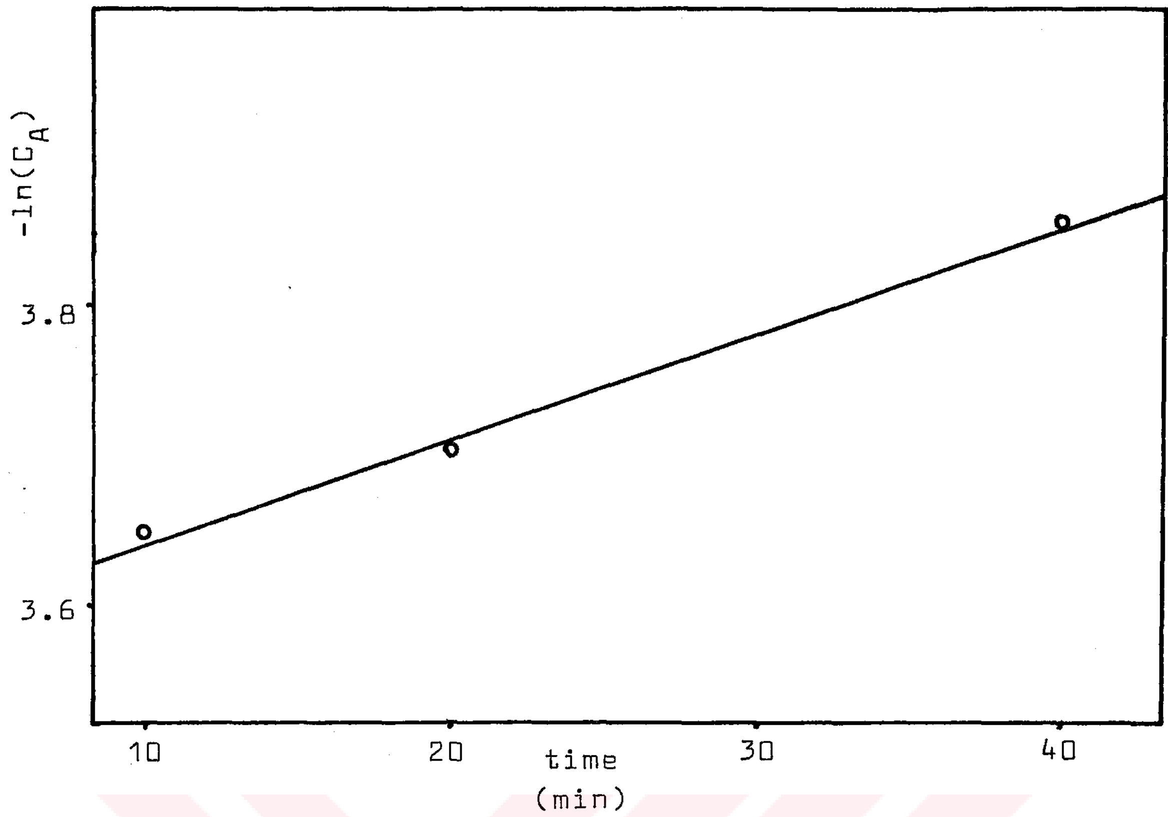



Figure 8. $-\ln(C_A)$ versus time for thiamin at 323 K

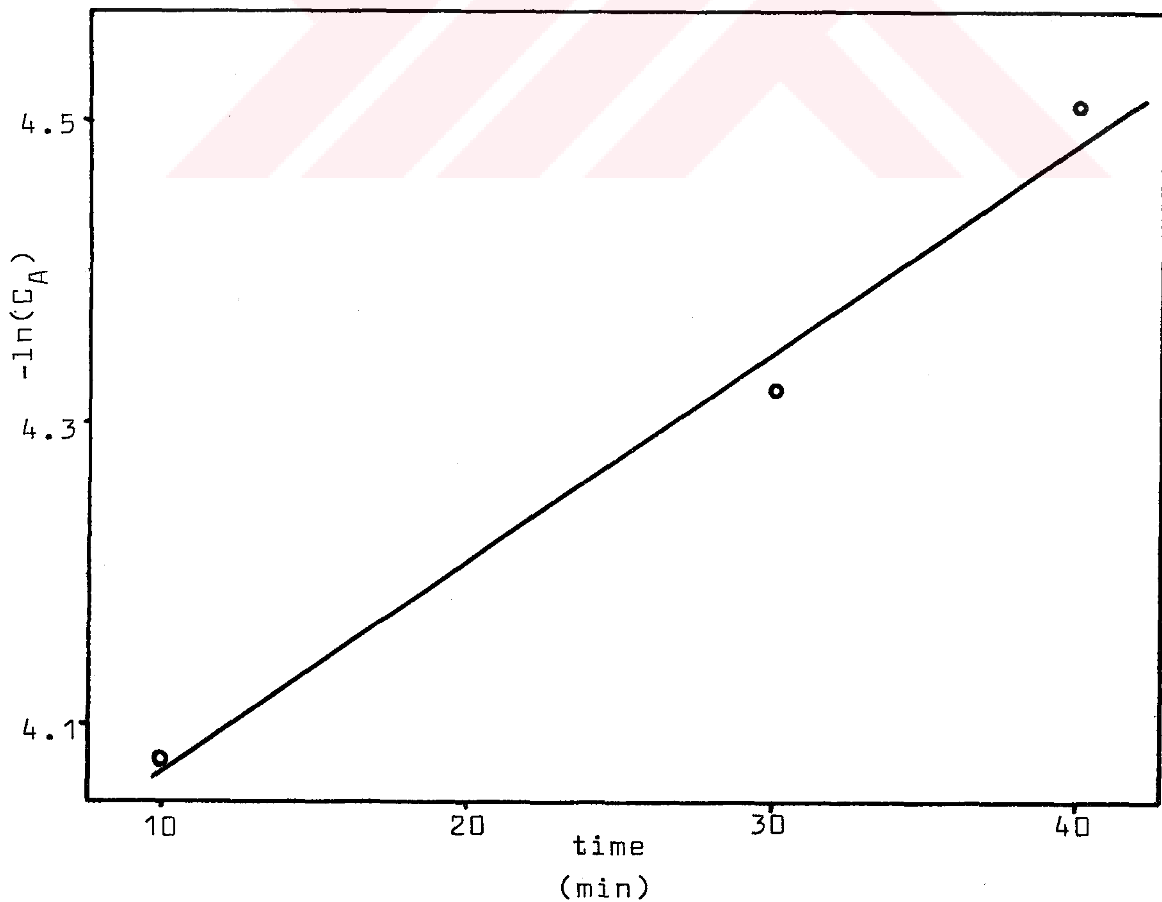



Figure 9. $-\ln(C_A)$ versus time for thiamin at 348 K.

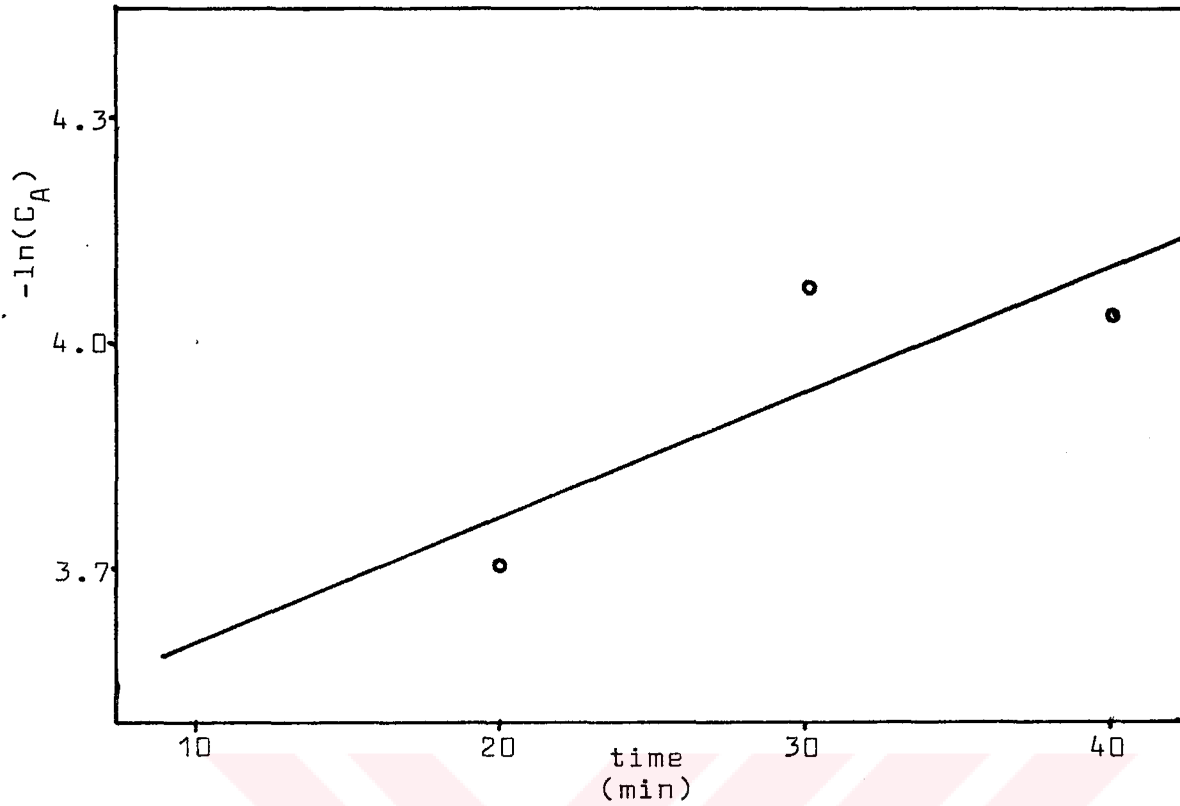



Figure 10. $-\ln(C_A)$ versus time curve for thiamin at 353 K.

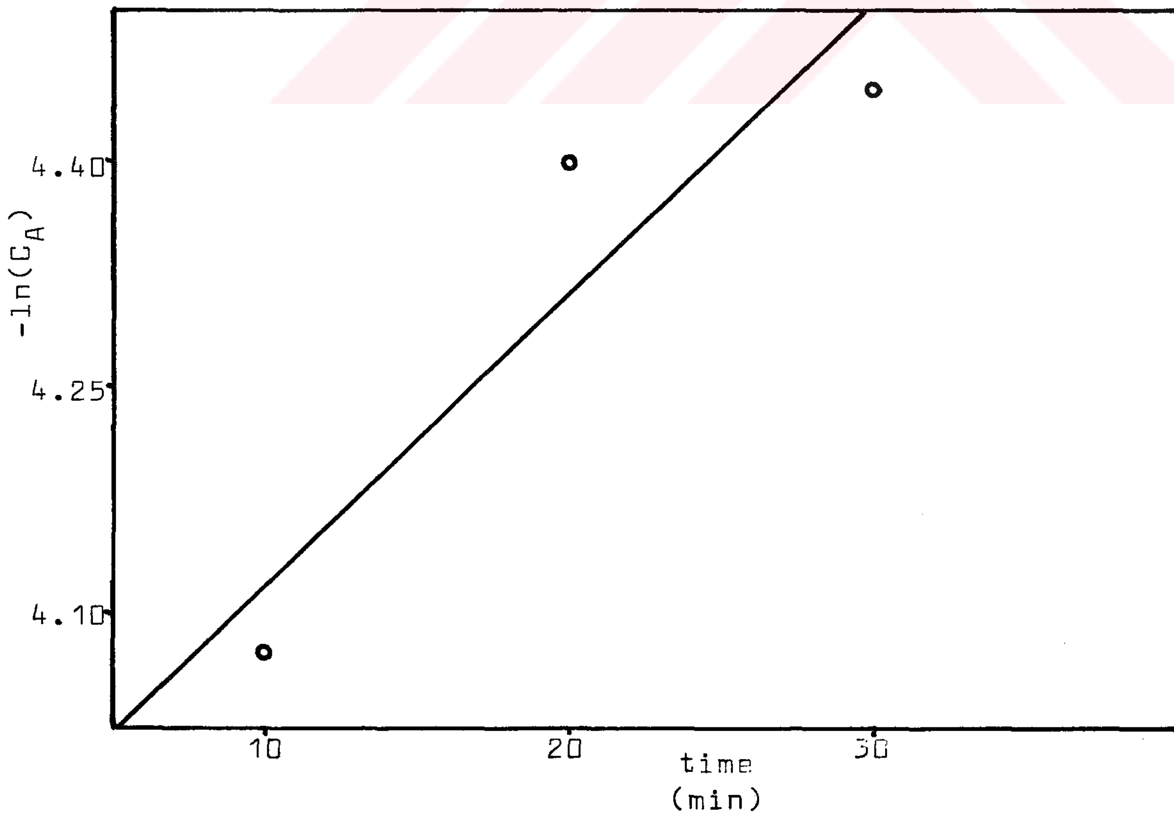
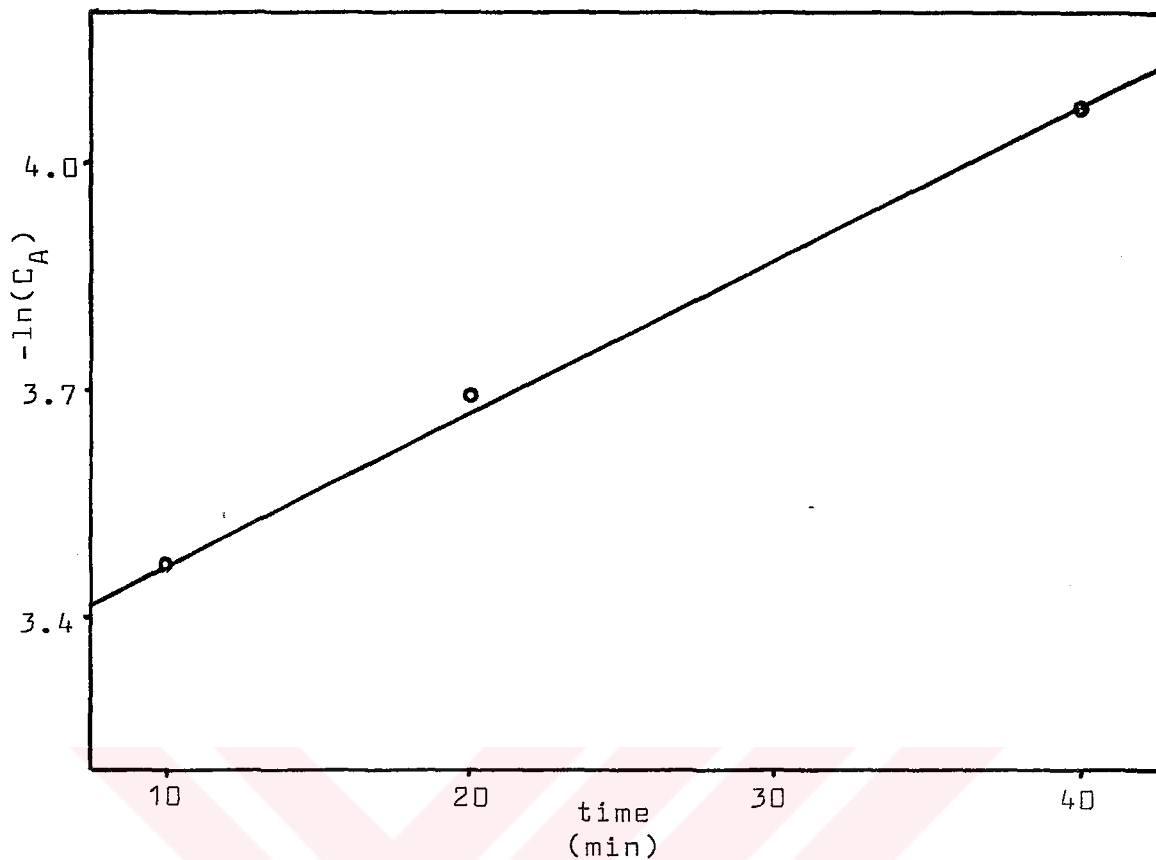



Figure 11. $-\ln(C_A)$ versus time curve for thiamin at 358 K.




Fgure 12. $-\ln(C_A)$ versus time curve for thiamin at 363 K.

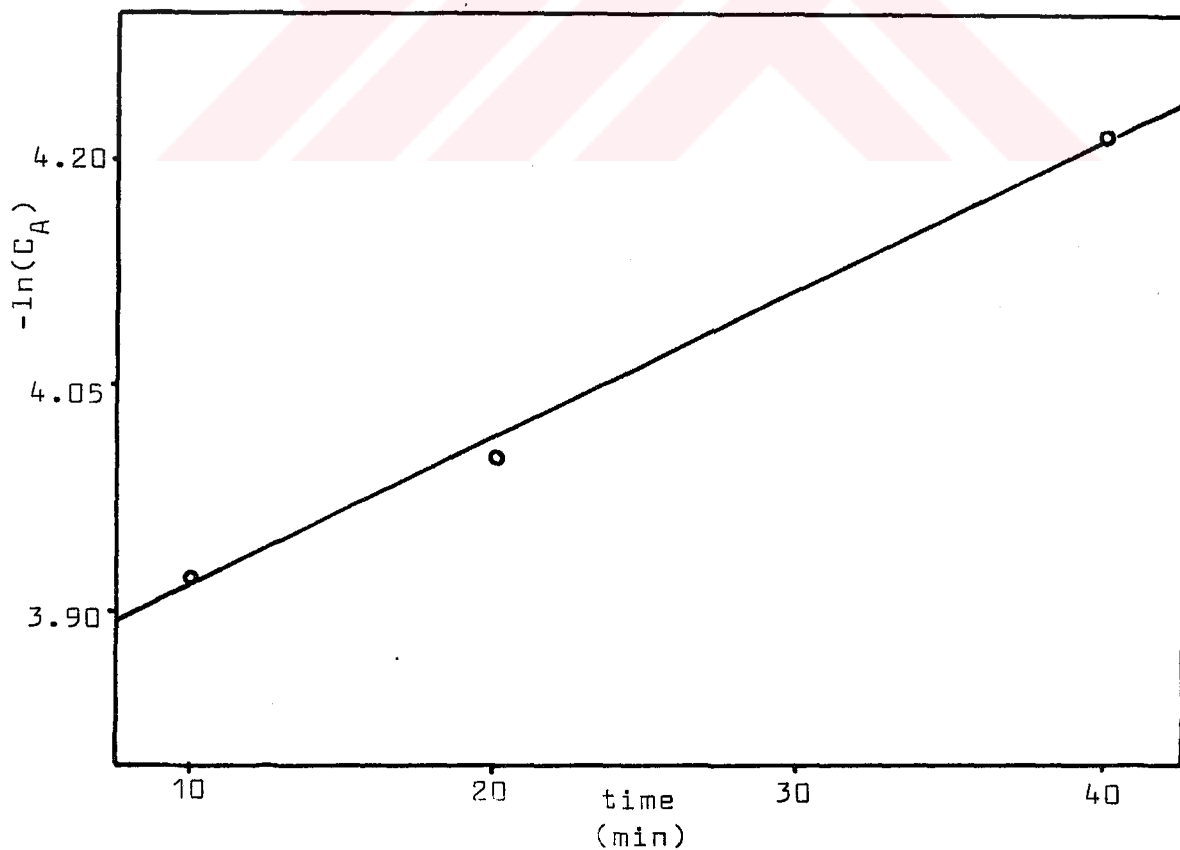



Figure 13. $-\ln(C_A)$ versus time curve for niacinamide at 323 K.

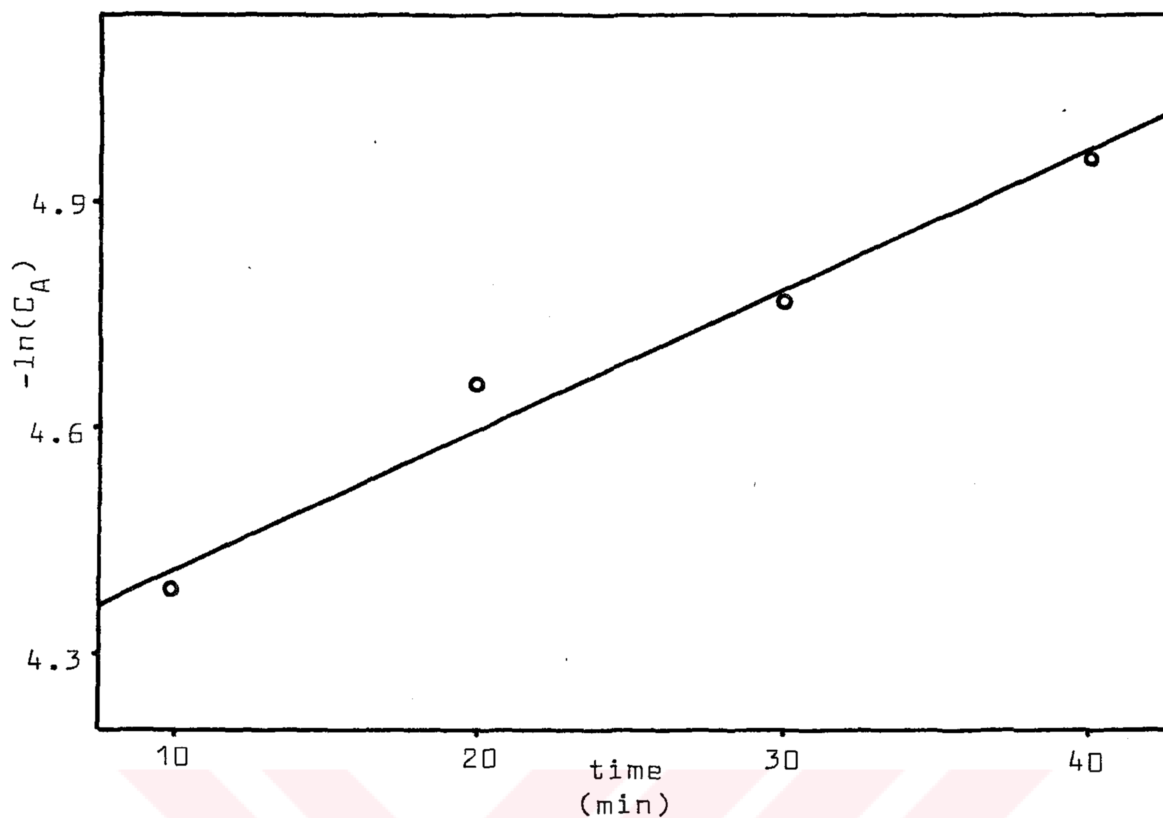



Figure 14. $-\ln(C_A)$ versus time for niacinamide at 348 K

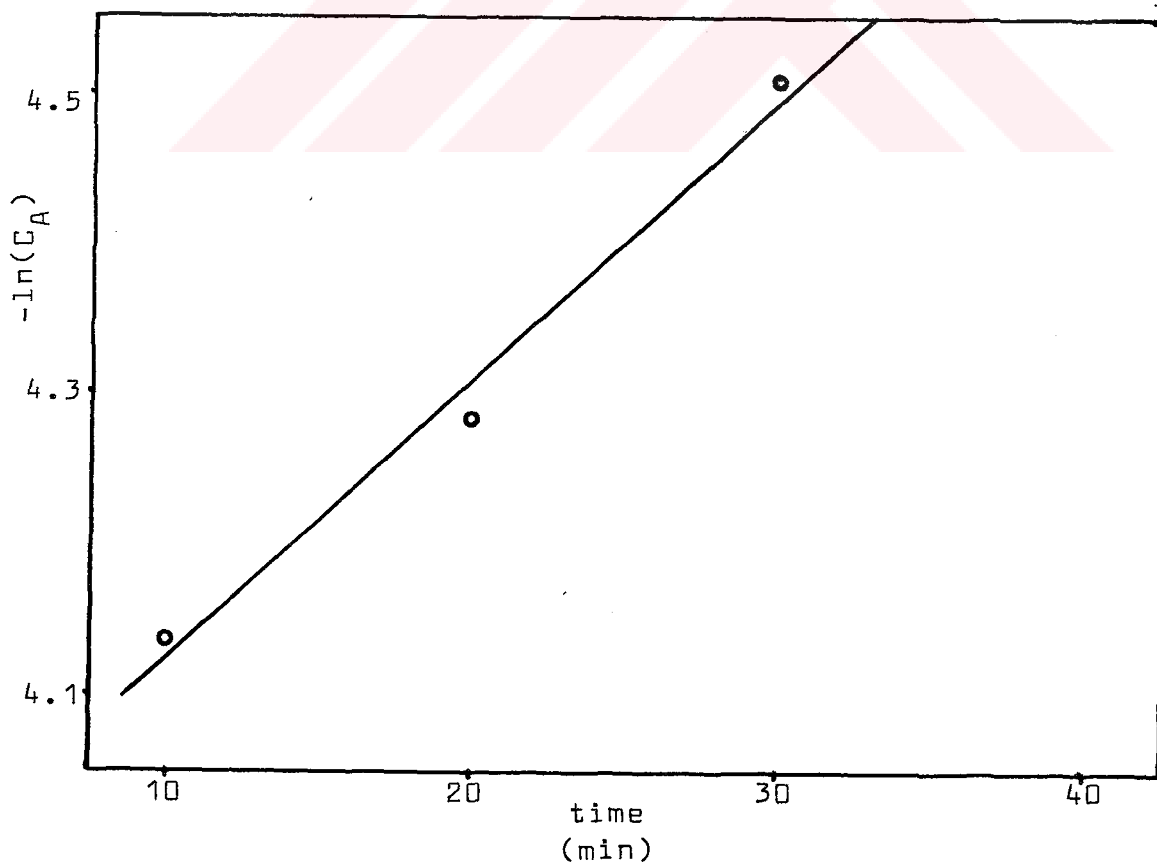



Figure 15. $-\ln(C_A)$ versus time curve for niacinamide at 353 K.

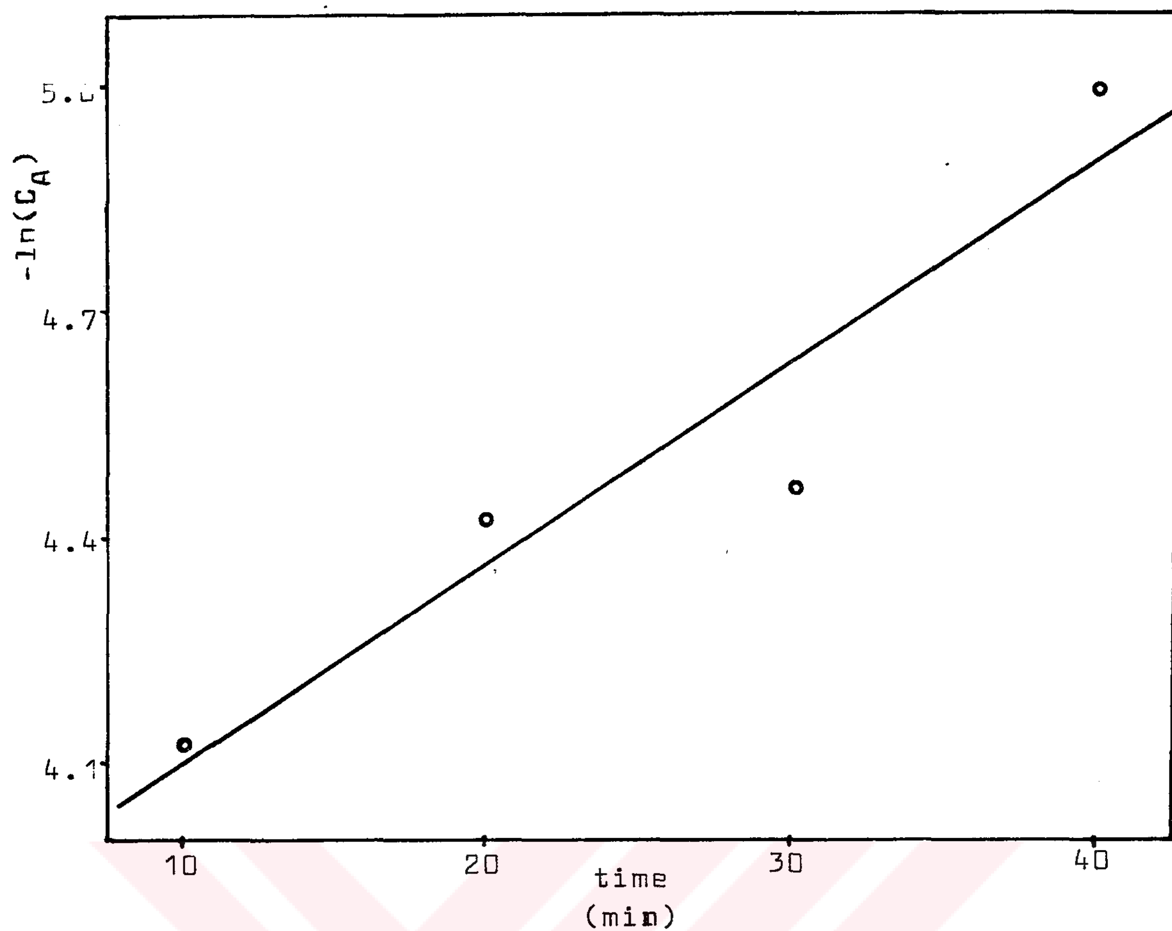



Figure 16. $-\ln(C_A)$ versus time curve for niacinamide at 363 K.

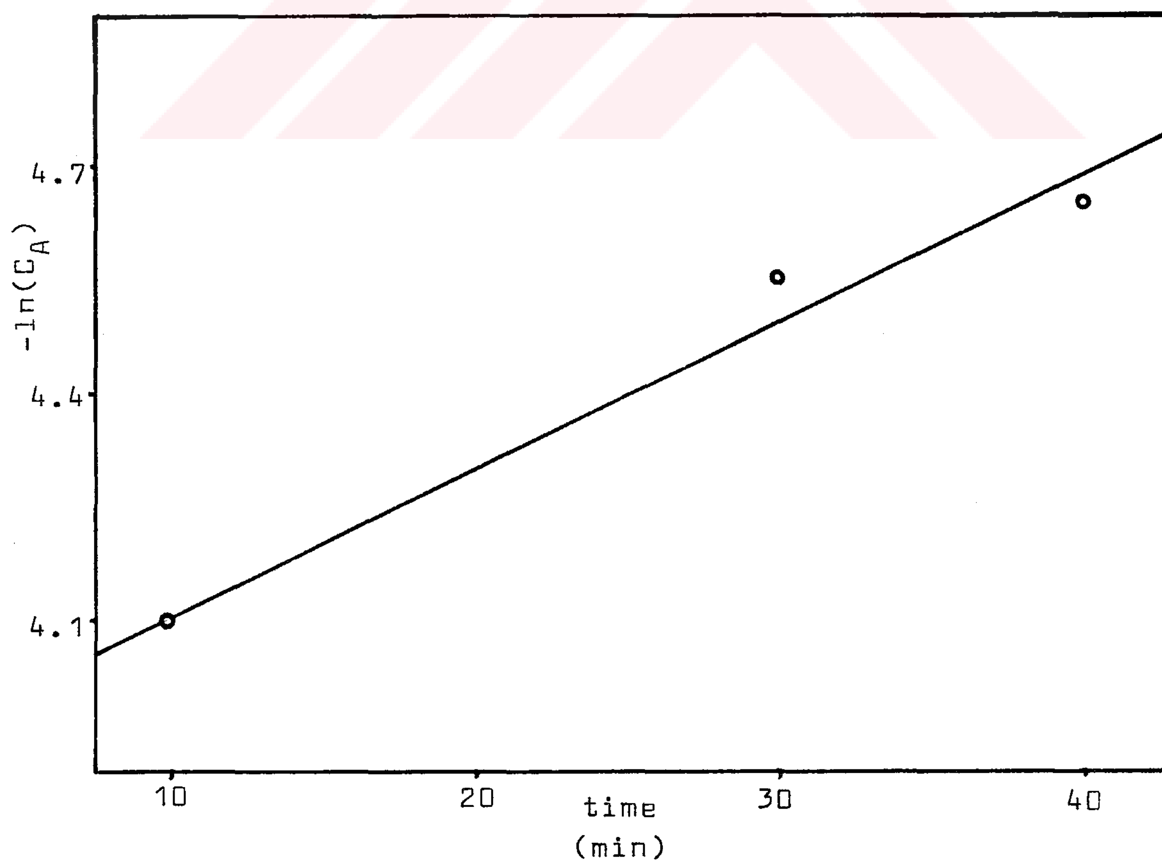



Figure 17. $-\ln(C_A)$ versus time curve for riboflavin at 323 K.

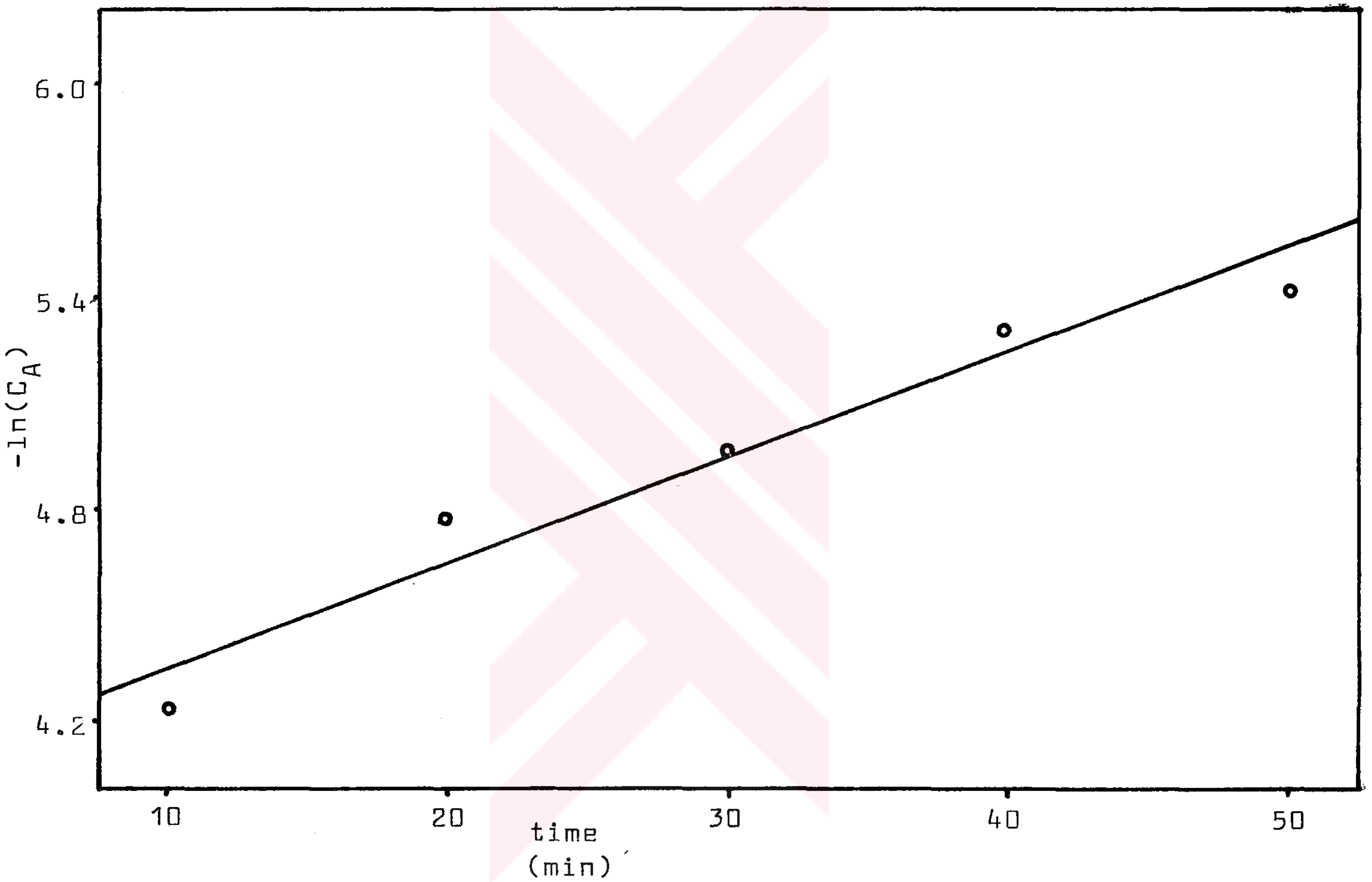



**Figure 18.** $-\ln(C_A)$ versus time curve for riboflavin at 348 K.

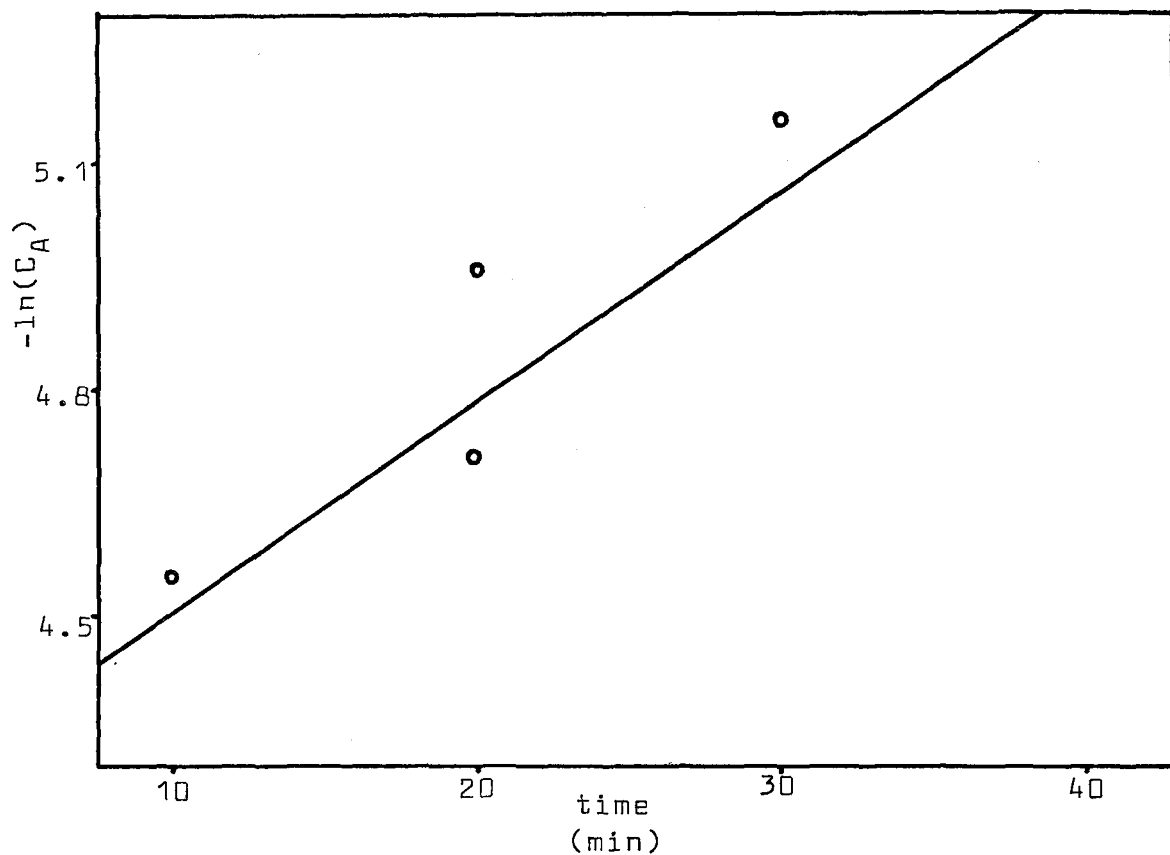



Figure 19. $-\ln(C_A)$ versus time curve for riboflavin at 353 K.

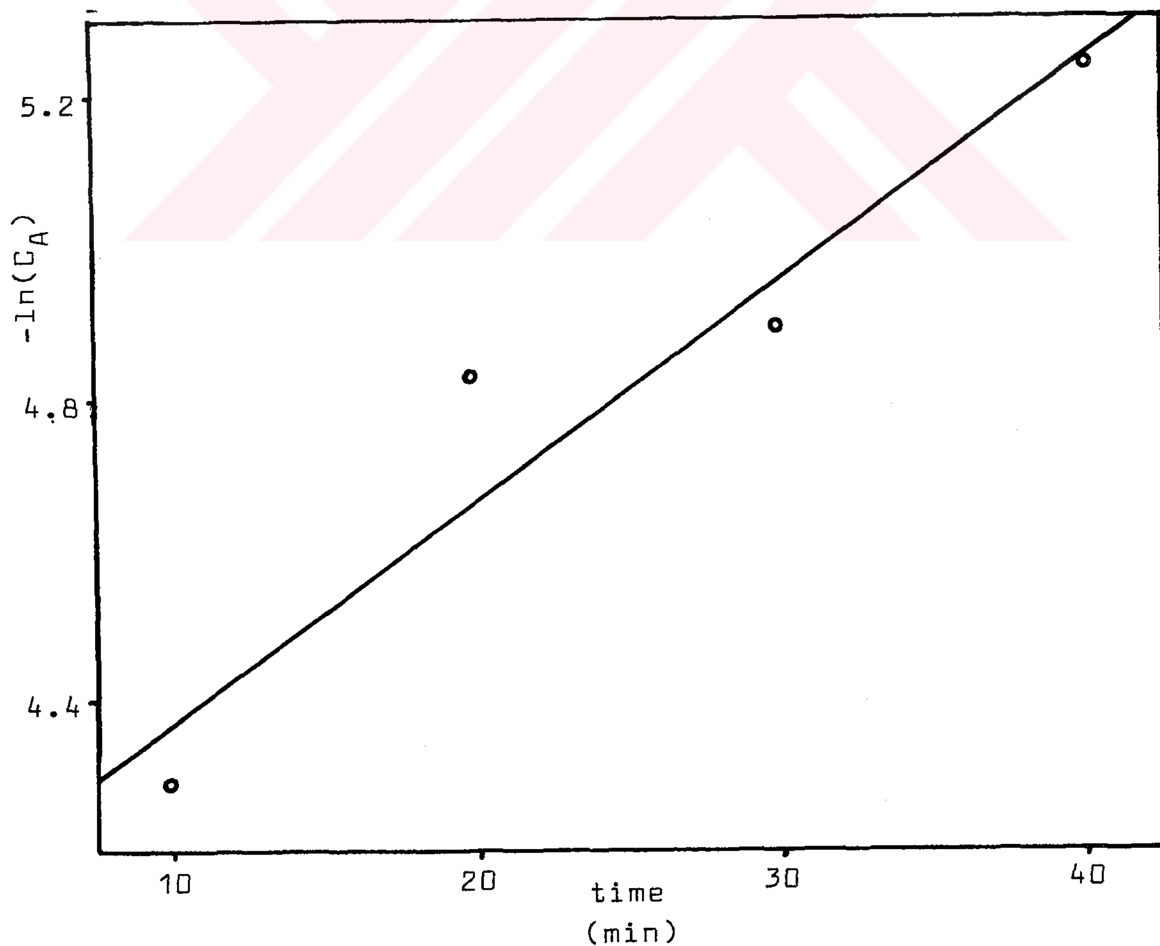



Figure 20. $-\ln(C_A)$ versus time curve for riboflavin at 363 K.

constants versus 1/T regressed to give the frequency factor (A) and the activation energy ($E_a$). ln of initial concentrations ($\ln(C_{Ao})$), rate constants (k) and regression coefficients ($r^2$) of thiamin, niacinamide and riboflavin at each temperature were shown in table 8.

**Table 8. Data Derived from Concentration -Time Information of Vitamins**

| Vitamin | $(1/T)\times10^3$ ($K^{-1}$) | $-\ln(C_{Ao})$ | k($min^{-1}$) | -lnk | $r^2$ |
|---|---|---|---|---|---|
| Thiamin | 3.10 | 3.574 | 0.0071 | 4.95 | 0.99 |
| | 2.87 | 3.924 | 0.0142 | 4.25 | 0.99 |
| | 2.83 | 3.433 | 0.0168 | 4.08 | 0.82 |
| | 2.79 | 3.924 | 0.0195 | 3.94 | 0.93 |
| | 2.75 | 3.261 | 0.0202 | 3.90 | 0.99 |
| Niacinamide | 3.10 | 3.819 | 0.0099 | 4.62 | 0.99 |
| | 2.87 | 4.229 | 0.0185 | 3.99 | 0.98 |
| | 2.83 | 3.936 | 0.0187 | 3.98 | 0.99 |
| | 2.75 | 3.836 | 0.0266 | 3.62 | 0.95 |
| Riboflavin | 3.10 | 3.932 | 0.0190 | 3.96 | 0.99 |
| | 2.87 | 4.072 | 0.0287 | 3.55 | 0.97 |
| | 2.83 | 4.234 | 0.0280 | 3.58 | 0.97 |
| | 2.75 | 4.077 | 0.0295 | 3.52 | 0.96 |

ln of k versus 1/T were plotted for thiamin in figure 21, for niacinamide in figure 22 and for riboflavin in figure 23.

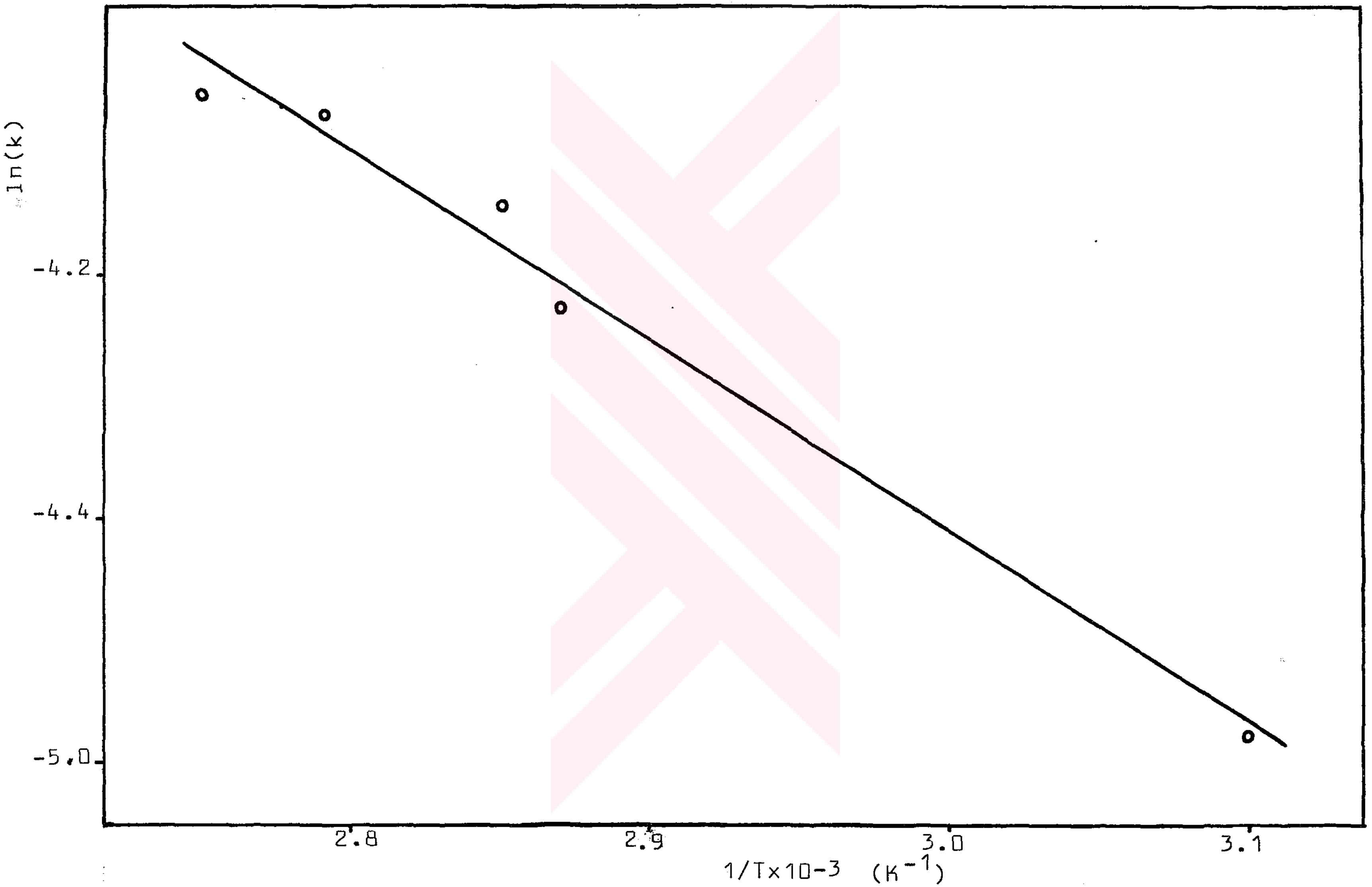



**Figure 21.** ln(k) versus 1/T curve for thiamin

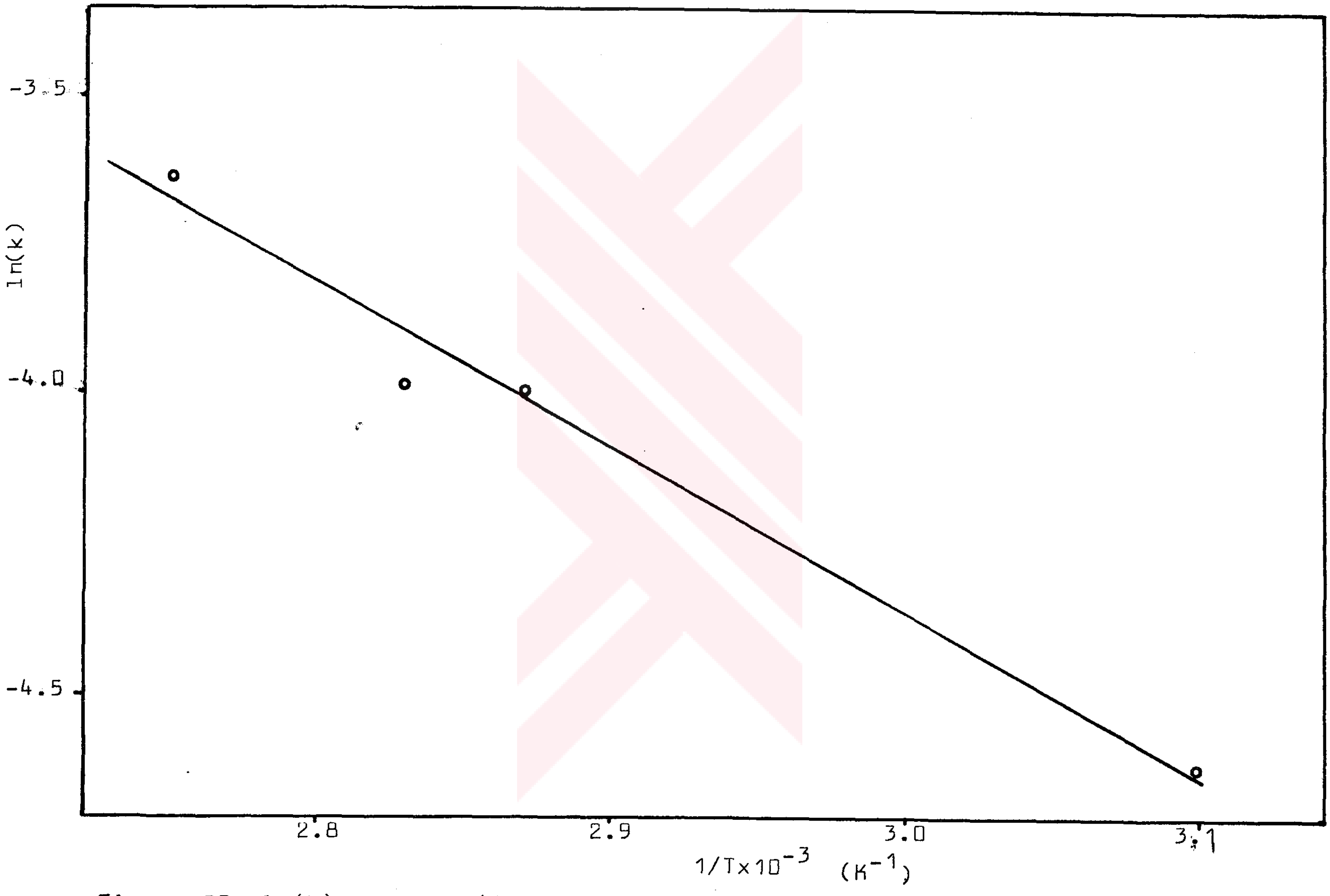



**Figure 22.** ln(k) versus 1/T for Niacinamide

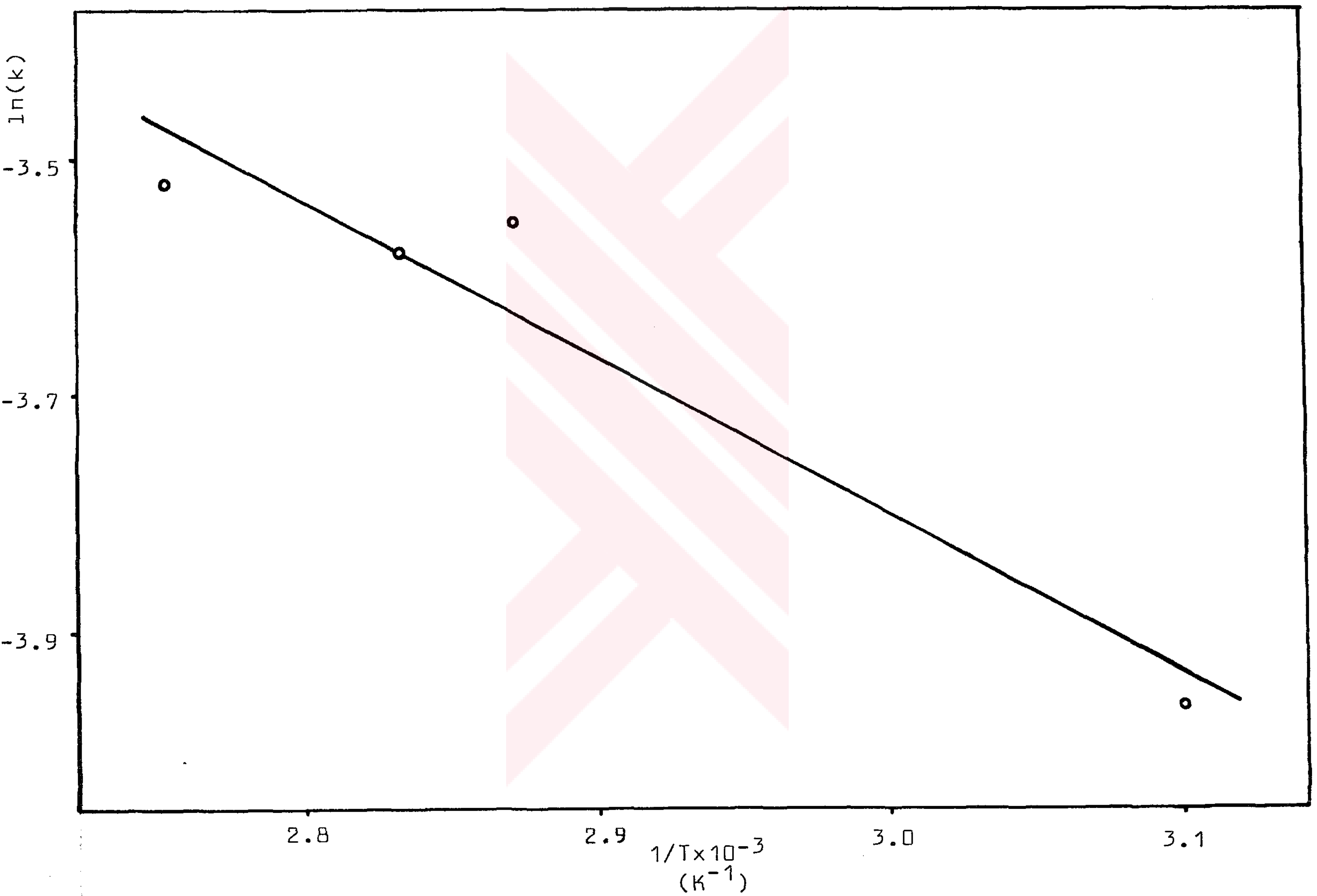



**Figure 23.** lnk versus 1/T curve for riboflavin.

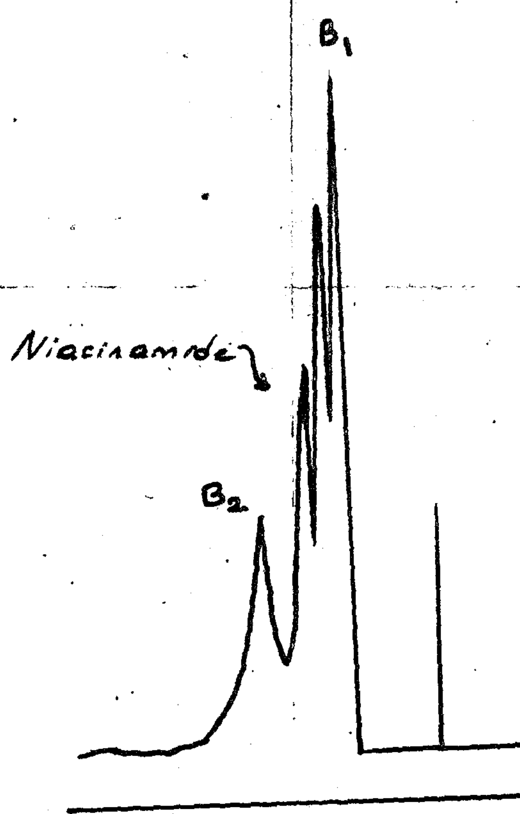
The activation energy ($E_a$), the frequency factor (A) in the form of ln(A), and regression coefficient for each vitamin were listed in table 9.

Table 9. Arrhenius Parameters for Thiamin, Niacinamide Riboflavin

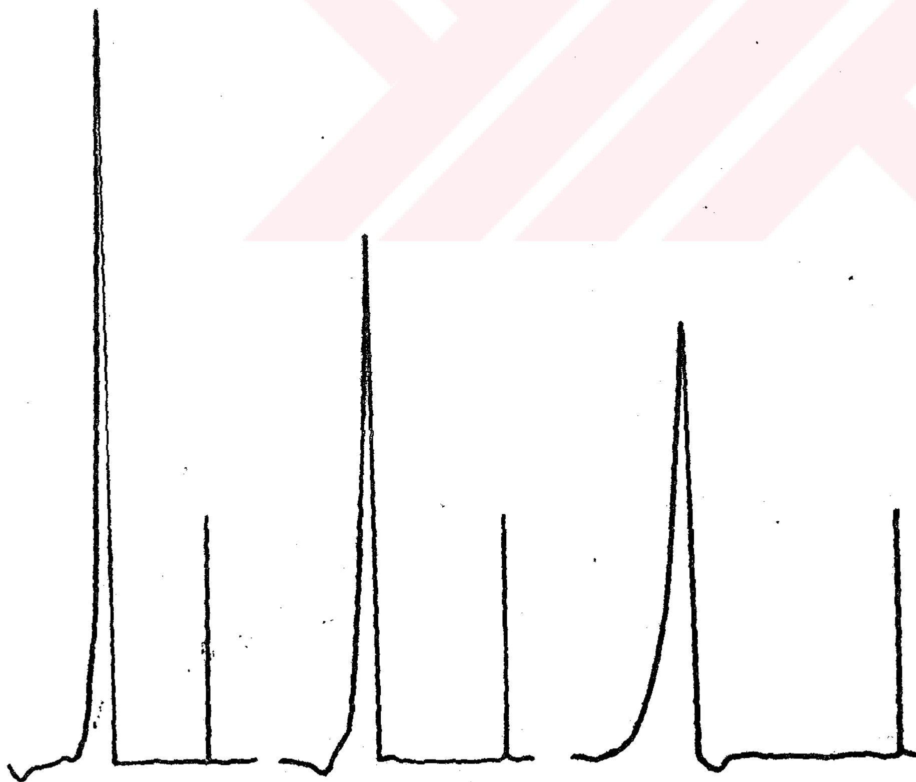
| Vitamin | ln(A) | $E_a$ ($kJ.mol^{-1}$) | $r^2$ |
|---|---|---|---|
| Thiamin | 4.743 | 25 ± 3.0 | -0.997 |
| Niacinamide | 3.823 | 22 ± 2.0 | -0.999 |
| Riboflavin | 0.171 | 11 ± 1.5 | -0.961 |

The error limits shown for activation energies are the estimates obtained by consideration of all possible experimental uncertainties during analysis. The activation energy obtained for riboflavin loss is in acceptable agreement with literature data. Activation energies for thiamin destruction in foods are generally reported to be much larger than that was obtained in this study. Activation energy calculated for niacinamide loss, on the other hand, seems to be in excellent agreement with single literature datum. These results will be discussed in detail in the following chapter.

Examples for the chromatograms of standard vitamin solutions and cooked macaroni samples are shown on the next page.




Chromatogram of macaroni sample




Chromatogram of standard vitamins

# CHAPTER 5

# DISCUSSION

## 5.1 General Results of The Kinetic Analysis

Engineers require quantitative models to design and optimize processes. In the food industry, these process models become very complex, because of the unique physical/chemical characteristics and variability of the raw material. Analysis of data describing rates of reactions and/or changes in food becomes very important.

Order of reaction can be determined by many different methods which were summarized in chapter 2. However, the time dependence for the majority of the losses in food appears to be described by zero or first order models [44] . Generally, loss of vitamins, like many other deteriorative reactions in foods was represented by a first order model. Although, there are some deviations from linearity which were observed on ln(C) versus time graph because mechanism of many such reactions are very complex, many scientists prefer using first order mechanism [37].

In this study, the kinetics of losses of thiamin, niacinamide and riboflavin during cooking procedures were assumed to follow first order reaction kinetics.

The figures showing the graphs obtained by regressing ln of concentration of vitamins versus time verified the first order assumption.

During the cooking of macaroni there are two factors that should be considered. One of them is the loss of vitamin due to the application of high temperature, the other is the leaching of vitamin into cooking water.

The parameters which affect the stability of thiamin are pH, temperature, ionic strength and other reacting species. Thiamin is converted to thiochrome, if it is oxidized under alkali conditions. It is also affected by strong nucleophiles, such as sulfite ion. Decomposition of thiamin takes place at high temperatures, about 473 K. Therefore, leaching into water is the most important factor which causes the loss of vitamin while the macaroni is cooked.

The activation energy, 25 kJ $mol^{-1}$, which is obtained in this study for thiamin, represents the activation energy for the loss of this vitamin by leaching.

Riboflavin, as mentioned in section 1.3.2 is more heat stable than thiamin but is affected by UV or visible light, yielding lumiflavin. In this study, all experiments were performed under subduedlight so the light factor was eliminated. Riboflavin is more stable under acid than under alkaline conditions. It is reported that this vitamin is quite stable in food under ordinary conditions. However, the trimming, milling and leaching operations cause losses of riboflavin in an extent similar to that of other water soluble vitamins. Decomposition of

riboflavin takes place at about 553 K with melting.

Therefore, the activation energy, 11 kJ $mol^{-1}$, found in this study corresponds to activation energy for the loss of riboflavin by leaching into water.

Niacinamide is reported to be the most stable vitamin among other B vitamins [45]. It is also lost during certain food preparation processes such as trimming and leaching. Both niacinamide and niacin are resistant to heat, acid or alkali conditions. Melting point of niacinamide is in the range of 401-404 K and that of niacin is at 510 K. As in the other B vitamins studied, the activation energy of 22 kJ $mol^{-1}$ obtained in this work should be taken as the activation energy for the loss of niacinamide through leaching process.

The solubilities of thiamin mononitrate, riboflavin and niacinamide are 0.03 $g.ml^{-1}$ $H_2O$, 0.07 $g.ml^{-1}$ $H_2O$ and 1.00 $g.ml^{-1}$ $H_2O$, respectively. These values are most probably the solubilities at 298 K. So as the temperature is increased, increased solubilities are expected. However, the solubility, in general, is not a direct function of temperature since it may be affected by several factors.

The extraction of a soluble constituent from a solid, by means of a solvent is generally referred to as leaching [46]. Leaching is a mass-transfer phenomena [47]. The process of leaching is not dependent only to

solubility but, the diffusion process of particles should also be considered. This is appearent from the values of activation energies calculated in this study for the three vitamins. If the solubility was the only rate determining factor, the activation energies should increase in the order of niacinamide, thiamin and riboflavin. However, the activation energy for thiamin was found to be larger than that of riboflavin. This can only be explained by considering the existence of other processes together with the solubility process during leaching phenomena.

The phenomenon of leaching includes both the extraction of the soluble compound from the substance added into the solvent and the diffusion of this compound within the solvent.

Extraction of the soluble compound becomes easier as the size of the compound gets smaller. Also, the viscosity of solvent plays an important role in the extraction process since the solvent with low viscosity may increase the rate of extraction by circulating freely around the substance. The concentration of the solution will also be different at different parts, being more at parts closer to the surface of the substance and less at parts far away from the substance. Since the solubilized compounds will be closer to the substance from which they are extracted, there will be a layer of these solubilized

compounds which will decrease the rate of more extraction. In general, as the temperature is increased, there will be more extraction due to the increased solubility.

The second process in leaching is diffusion. Although the diffusion process is another area of interest, a brief explanation related to the phenomena of leaching might be necessary here. Because the diffusion process is also a rate process, it occurs at a finite rate. Since the diffusion of particles in a solution occurs, if there is a concentration difference in the solution, it is related also to the rate of extraction of these particles.

The change in the concentration of a diffusing particle by time, the rate of diffusion, can be expressed by Fick's second law:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \qquad 5.1$$

where x represents the distance and D is the diffusion coefficient.

The diffusion coefficient D is related to the jump frequency of the particle, k, by the equation

$$D = \frac{1}{2} l^2 k \qquad 5.2$$

l here is the main jump distance that the particle can move between successive jumps. During the diffusion of a particle, the structure of the solvent and the

interatomic forces which operate in the medium are important. Therefore, depending on these factors, the mean jump distance and the jump frequency change. It is accepted that the particle jumps from an empty site in the structure of the solvent to the other during diffusion. This can be similarized to the changes occurring in the places of atoms in chemical reactions. A particle in a chemical compound may jump from its original place to another which is now next to a different particle. If this is assumed, then, there is an analogy between the two rate processes, i.e., diffusion and chemical reaction. Therefore, the basic theory of rate processes, the so-called Transition State Theory, can also be applied to the proces of diffusion.

In this theory, it is accepted that as the particles move to complete the process, the potential energy (and standard free energy) of the system changes. Most of the time, the movements crucial to the process are those of a single particle, as is the case with the diffusive jumps of a particle from site to site. In order for the process to be accomplished, the system should attain a certain energy, called Gibbs free energy of activation, $\Delta G^{\neq}$. Therefore, it means that the system should be able to pass over an energy barrier, if the reaction should occur. The rate constant of the reaction can be expressed in terms of this $\Delta G^{\neq}$ from a thermodynamical point of

view as in the following equation

$$k = \frac{kT}{h} e^{-\Delta G^{\neq}/RT} \qquad 5.3$$

where k is the Boltzmann constant and h is the Planck's constant.

The rate constant, in equation 5.3 is also the jump frequency k, in the case of diffusion. If the long derivations are omitted, the thermodynamic equation for a first order rate constant can be written as

$$k = e \frac{kT}{h} e^{\Delta S^{\neq}/R} e^{-E_a/RT} \qquad 5.4$$

where $\Delta S^{\neq}$ represents the entropy change of activation.

The values of entropy of activation $\Delta S^{\neq}$, Gibb s free energy of activation $\Delta G^{\neq}$, and enthalpy of activation, $\Delta H^{\neq}$, calculated from $E_a = \Delta H^{\neq} + RT$ for each of the vitamins are given in table 10.

**Table 10 . Thermodynamics Data For Thiamin, Niacinamide and Riboflavin**

| Vitamin | $\Delta S^{\neq}$ ($J.mol^{-1}K^{-1}$) | $\Delta G^{\neq}$ ($kJ.mol^{-1}$) | $\Delta H^{\neq}$ ($kJ.mol^{-1}$) |
|---|---|---|---|
| Thiamin | -215.770 | 104.287 | 22.945 |
| Niacinamide | -223.060 | 103.654 | 19.665 |
| Riboflavin | -253.430 | 103.018 | 8.005 |

There is no such thermodynamic data reported for thiamin, niacinamide and riboflavin, so no comparison can be made here.

The activation energies calculated in this study, actually result from combination of many factors. Solubilities of vitamins, extraction of vitamins from macaroni in water and diffusion of vitamins in water seem to be the most important factors. The ease of extraction of each vitamin from macaroni may be different, if the compound bound to each vitamin and the protective compounds around vitamin molecules are considered. However, there are some unknowns in these respects, particularly the degree of protective action of compounds surrounding these vitamins is not very certain.

For example, the stability of thiamin is so strongly influenced by the nature and state of the system, that it is difficult to extrapolate between systems, and numerious unexplainable differences between similar investigations exist in the scientific literature [45]. Mulley [37] suggested that starch in macaroni prevent the heat destruction of thiamin to a great extent.

As a result of these discussions about the extraction and diffusion in leaching process, it can be said that the activation energies obtained in this study correspond to the energies that the vitamins should acquire in order for the leaching process to take place.

The activation energies for thiamin, niacinamide and riboflavin were calculated as 25, 22 and 11 $kJ.mol^{-1}$, respectively as written in section 4.3. It seems that the leaching of thiamin from macaroni samples in water in the temperature range of (323-363 K) has the largest activation energy indicating the difficulty of extraction and diffusion process for this vitamin in the experimental conditions.

Riboflavin extraction and leaching occurs most easily and these processes takes place for niacinamide with an activation energy closer to that of thiamin.

Analyses also showed that the concentration of these vitamins in macaroni are in the increasing order for niacinamide, riboflavin and thiamin. This also indicates that when the concentration of vitamin is large in macaroni, the first layer of dissolved vitamin in macaroni-water solution strongly resricts the further extraction of this vitamin.

## 5.2 Analysis of The Cooking Waters of Macaroni

In this study, aside from the analysis of the macaroni samples, the cooking waters were also analyzed for their vitamin contents. However, there were some difficulties in the treatments of these data. One of them was the error in concentrations of vitamins due to the evaporation of water during cooking, and the second difficulty

was the measurements of the peak heights during chromatographic analysis under certain conditions.For this reason, the experimental data of the cooking water analysis were not given in chapter 4. However, in order to find the volume of the evaporated water at different temperatures and different cooking times, similar experiments were repeated but without any analysis.

As a result,correction factors for the volumes were obtained for each experiment and the sum of the concentration in the cooked macaroni samples and the corrected concentration in cooking waters was compared with the calculated initial total concentration ($C_{Ao}$) of each vitamin in macaroni. It is clear that such a comparison will contain large errors, especially due to correction factors. Therefore, these data were not given in a table. However, it was observed that in many cases, there were very close agreements between the calculated initial concentrations of the vitamins and the sum of these vitamins in cooking water and in macaroni samples. For example, calculated $C_{Ao}$ for niacinamide at 323 K was 0.022 $mg.ml^{-1}$ and the sum of the concentration of niacinamide was 0.0218 $mg.ml^{-1}$ after 40 minutes cooking. For riboflavin, $C_{Ao}$ was 0.0145 $mg.ml^{-1}$ at 353 K while the sum was 0.0146 $mg.ml^{-1}$ after 20 minutes heating and 0.0145 $mg.ml^{-1}$ after 30 minutes heating.

These examples illustrate that the losses of vitamins

under the experimental conditions only take place as a result of leaching into water.

## 5.3 Percent Loss of Vitamins at Different Temperatures

The experimental data obtained in this study were also treated to calculate the percent loss of each of the vitamin at the temperatures studied. Concenration of each of the vitamin retaining in the cooked macaroni after different heating times and the initial concentrations of regression analysis of data were used for this purpose.

The calculated initial concentrations, $C_{Ao}$ values and calculated percent losses of thiamin, niacinamide and riboflavin were shown in the tables 11-13.

As it is seen from these tables, as the temperature is increased percent loss of each of the vitamin increases depending on the increased heating times. Thiamin losses are small at first, at low temperatures but riboflavin is lost rapidly from macaroni during the early stages of cooking. Niacinamide shows an intermediate behaviour between thiamin and riboflavin. At the highest temperatures of cooking, thiamin showed 55.61 percent loss, riboflavin and niacinamide showed 69.04 and 68.72 percent losses at the longest time of heating. These observations are in accordance with the activation energies calculated for these vitamins. Activation energies

Table 11. Percent Loss of Thiamin Under Different Cooking Conditions

| T (K) | $C_{Ao}$ ($mg.ml^{-1}$) | time (min) | $C_A$ ($mg.ml^{-1}$) | lost (%) |
|---|---|---|---|---|
| | | 10 | 0.0260 | 7.15 |
| 323 | 0.0280 | 20 | 0.0245 | 12.50 |
| | | 40 | 0.0210 | 25.00 |
| | | 10 | 0.0170 | 13.96 |
| 348 | 0.0198 | 30 | 0.0130 | 34.21 |
| | | 40 | 0.0110 | 44.33 |
| | | 20 | 0.0245 | 24.10 |
| 353 | 0.0323 | 30 | 0.0170 | 47.33 |
| | | 40 | 0.0175 | 45.78 |
| | | 10 | 0.0170 | 13.96 |
| 358 | 0.0198 | 20 | 0.0123 | 37.75 |
| | | 30 | 0.0115 | 41.80 |
| | | 10 | 0.0310 | 19.06 |
| 363 | 0.0383 | 20 | 0.0260 | 32.11 |
| | | 40 | 0.0170 | 55.61 |

Table 12. Percent Loss of Niacinamide Under Different Cooking Conditions

| T (K) | $C_{Ao}$ ($mg.ml^{-1}$) | time (min) | $C_A$ ($mg.ml^{-1}$) | lost (%) |
|---|---|---|---|---|
| 323 | 0.0220 | 10 | 0.0198 | 9.82 |
| | | 20 | 0.0183 | 16.66 |
| | | 40 | 0.0148 | 32.64 |
| 348 | 0.0146 | 10 | 0.0125 | 14.38 |
| | | 20 | 0.0095 | 37.78 |
| | | 30 | 0.0085 | 41.65 |
| | | 40 | 0.0070 | 52.05 |
| 353 | 0.0195 | 10 | 0.0160 | 18.08 |
| | | 20 | 0.0138 | 29.58 |
| | | 30 | 0.0110 | 43.66 |
| 363 | 0.0216 | 10 | 0.0162 | 24.92 |
| | | 20 | 0.0120 | 44.39 |
| | | 30 | 0.0115 | 46.71 |
| | | 40 | 0.0068 | 68.72 |

Table 13. Percent Loss of Riboflavin Under Different Cooking Conditions

| T (K) | $C_{Ao}$ ($mg.ml^{-1}$) | time (min) | $C_A$ ($mg.ml^{-1}$) | % lost |
|---|---|---|---|---|
| 323 | 0.0196 | 10 | 0.0165 | 15.81 |
| | | 30 | 0.0105 | 46.43 |
| | | 40 | 0.0095 | 51.53 |
| 348 | 0.0170 | 10 | 0.0145 | 14.71 |
| | | 20 | 0.0085 | 50.00 |
| | | 30 | 0.0070 | 58.82 |
| | | 40 | 0.0050 | 70.58 |
| | | 50 | 0.0045 | 73.53 |
| 353 | 0.0145 | 10 | 0.0105 | 27.58 |
| | | 20 | 0.0090 | 37.93 |
| | | 30 | 0.0060 | 58.62 |
| 363 | 0.0169 | 10 | 0.0138 | 18.96 |
| | | 20 | 0.0080 | 52.82 |
| | | 30 | 0.0075 | 55.77 |
| | | 40 | 0.0053 | 69.04 |

for thiamin was higher than those of riboflavin and niacinamide and thus, percent loss of thiamin is smaller than percent losses of other two vitamins.

## CHAPTER 6

## CONCLUSIONS

1. A rapid, accurate analysis technique was developed for the determination of thiamin, niacinamide and riboflavin in macaroni by using simultaneous High Pressure Liquid Chromatographic technique with µBondapak $NH_2$ column. The mobile phase used in this study was methanol-water (20:80, pH=3-4).
2. The activation energies for the losses of thiamin, niacinamide and riboflavin were found to be 25, 22 and 11 $kJ.mol^{-1}$, respectively.
3. Leaching is the main factor affecting the losses of vitamins in macaroni during cooking. It was supported by analyzing the vitamin content of the cooking water.
4. Thermodynamic data for thiamin, niacinamide and riboflavin were calculated by using Arrhenius parameters. $\Delta S^{\neq}$ was found as -215.770 $J.mol^{-1}.K^{-1}$ for thiamin, -223.060 $J.mol^{-1}.K^{-1}$ for niacinamide and -253.430 $J.mol^{-1}.K^{-1}$ for riboflavin. $\Delta G^{\neq}$ was found as 104.287 $kJ.mol^{-1}$ for thiamin, 103.654 $kJ.mol^{-1}$ for niacinamide and 103.018 $kJ.mol^{-1}$ for riboflavin. $\Delta H^{\neq}$ was found as 22.945, 19.665 and 8.005 $kJ.mol^{-1}$ for thiamin, niacinamide and riboflavin, respectively.
5. As the temperature is increased, percent loss of each

of the vitamin is also increased depending on the increased heating times. At the highest temperature and longest time of cooking thiamin, niacinamide and riboflavin showed 55.61, 69.04 and 68.72 percent losses, respectively.

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