CHAPTER 3

KINETIC MEASUREMENTS AND PRODUCT CHARACTERIZATIONS

3.1 Introduction

Chemical kinetics is concerned with the analysis of the dynamic of chemical reactions.[1-5]A chemical reaction is typically the transformation of one compound into another by one or more. The chemical kinetic is the study of reaction rates and their interpretation in terms of mechanism of reaction. The study of reaction mechanism includes four components: a) experimental kinetics, b) determination of the rate laws, c) writing the kinetics scheme/reaction mechanism and d) proposal of transition state structures.

In principle any property of a reacting system which changes as the reaction proceeds may be monitored in order to accumulate the experimental data which lead to determination of rate law, rate constant, kinetic isotope effect and etc. In order to determine the kinetic order of the reaction, several methods can be applied. a) The half-life method; the time required for one-half of the initial concentration of a given reactant to be consumed, $t_{1/2}$. b) Substitution effect- applicable to reactions that are not complex and k should remain constant throughout the course of the reaction.[4] c) The initial rate method involves measuring the rate of reaction, at very short times before any significant changes in concentration occur. This can be applied to obtain the rate constants of slow reactions. d) The isolation method- in this method the concentration of one reactant is made much smaller than the concentrations of the other reactants.

Model reactions are set up and the appropriate rate equations are written. If the kinetics of a complex reaction process is found to fit closely with the model equation derived, the model can be used as a basis for the mechanistic description of the process. If not, further refinement or elimination of approximations may lead to considerable modification of the description of a mechanism. Each complex reaction

contained a set of simple or elementary chemical reactions which, when combined, give a complex reaction. One of the elementary steps is rate-limiting or rate-determining steps, i.e. the one which principally limits the rate of the overall process.

The rates of chemical reaction are controlled by some factors which are a) volume b) pH c) solvent d) ionic strength and e) the temperature. Pseudo first-order-rate law is applied throughout the studies, it permits the simplification of the reaction kinetics. This also can be applied to complicated rate law to transform into a simpler reaction equation. Applying pseudo-first-order allowed us to ignore the exact concentration of the reactant or product which appears in first-order reaction. In term of product characterization of particular reaction, one can compare the observed extinction coefficients with that of the authentic expected product, or with the authentic product using other techniques such as HPLC, NMR and IR. In our case the product characterization was determined by HPLC will be discussed later.

3.2 Kinetic Measurements

3.2.1 Wavelength Determination for Spectrophotometric Kinetic Studies

3.2.1.1 Alkaline Hydrolysis of *p*-Nitrophenyl Acetate (26) in Mixed Aqueous - Organic Solvent

p-Nitrophenyl acetate (**26**) is not very soluble in water and our compound *N*,*N*-(diethylaminomethyl)benzyl alcohol (**35**) also is not very soluble in water, so the kinetic measurements were carried out in mixed water - acetonitrile. In order to determine suitable wavelength for the kinetic measurement of alkaline aqueous of **26** at different content of organic co-solvent, the UV spectra of **26** at 2 %, 50 % and 80 % v/v CH_3CN were recorded.

In a typical kinetic run with total volume of 5 ml of reaction mixture containing 0.5 ml of 0.01 M NaOH and 4.4 ml H_2O, the reaction mixture was allowed to temperature-equilibrate for ~ 15 minutes at 30 °C. The reaction was then initiated by adding 0.1 ml of 0.003 M of **26** (prepared in CH_3CN) to the temperature-equilibrated reaction mixtures. The resulting aqueous reactions mixture contained 2 % v/v CH_3CN. The UV spectra of reaction mixtures were scanned at different time intervals and are shown in Fig. 3-1. Similar spectra of reaction mixture at 50 % v/v and 80 % v/v CH_3CN was monitored at different time intervals and was shown in Figs. 3-2 and 3-3.

It is evident from Figs. 3-1 to 3-3, there are two major different changes in absorption with the increase of reaction time. First at 400 nm, where the absorbance was found to increase with the increase of reaction time due to formation of phenolate ion (**48**) and at 271 nm where absorbance (Abs) was found to decrease with the increase of the reaction time due to disappearance of reactant **26**. Although there are two different wavelengths, the wavelength at 400 nm was considered for the rate measurements.

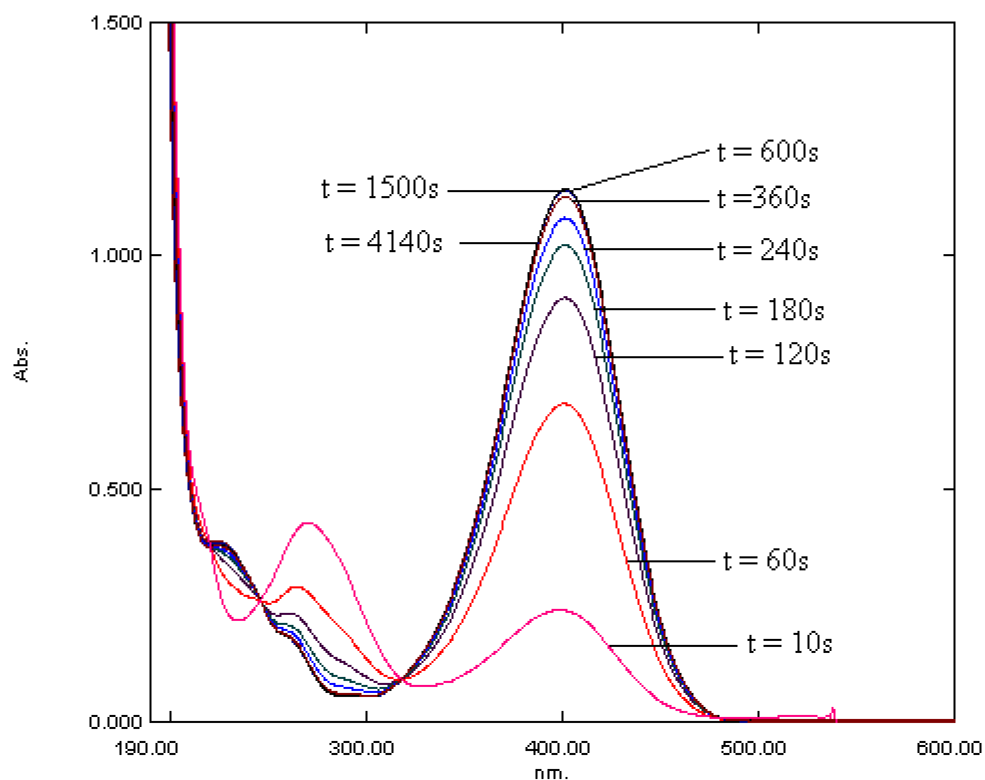


Figure 3-1: UV spectra of alkaline hydrolysis of **26** at 30°C at different reaction time (t) in aqueous solvent containing 6 x 10^{-5} M **26**, 1 x 10^{-3} M NaOH and 2 % CH_3CN.

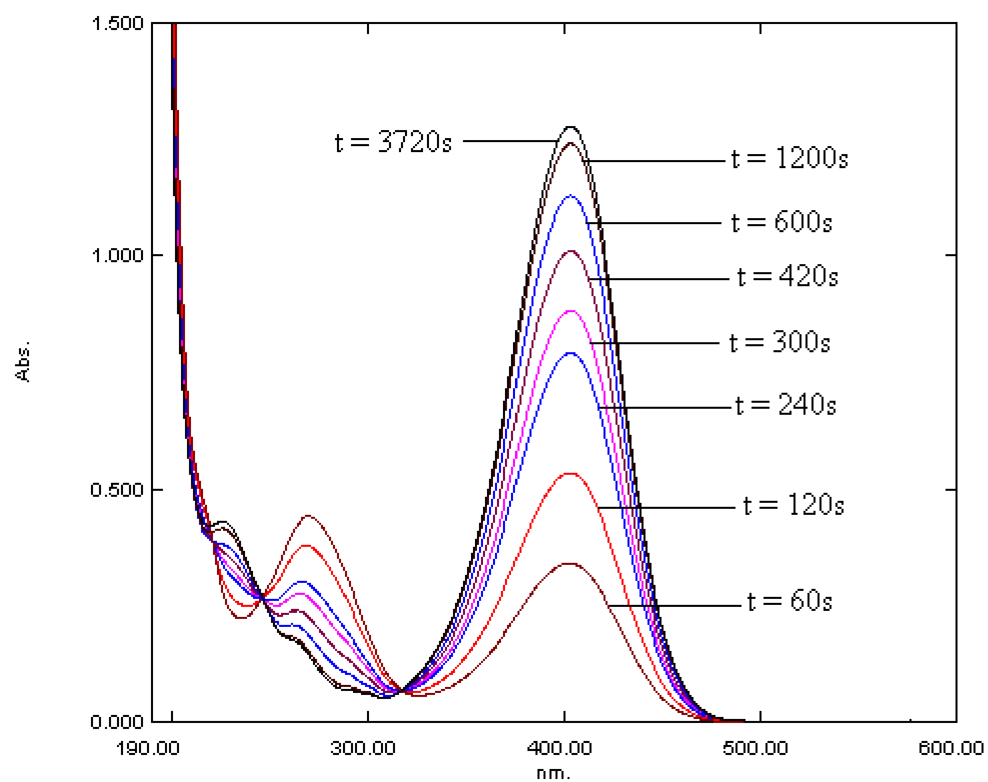


Figure 3-2: UV spectra of alkaline hydrolysis of **26** at 30°C at different reaction time (t) in aqueous solvent containing 6 x 10^{-5} M **26**, 1 x 10^{-3} M NaOH and 50 % CH_3CN.

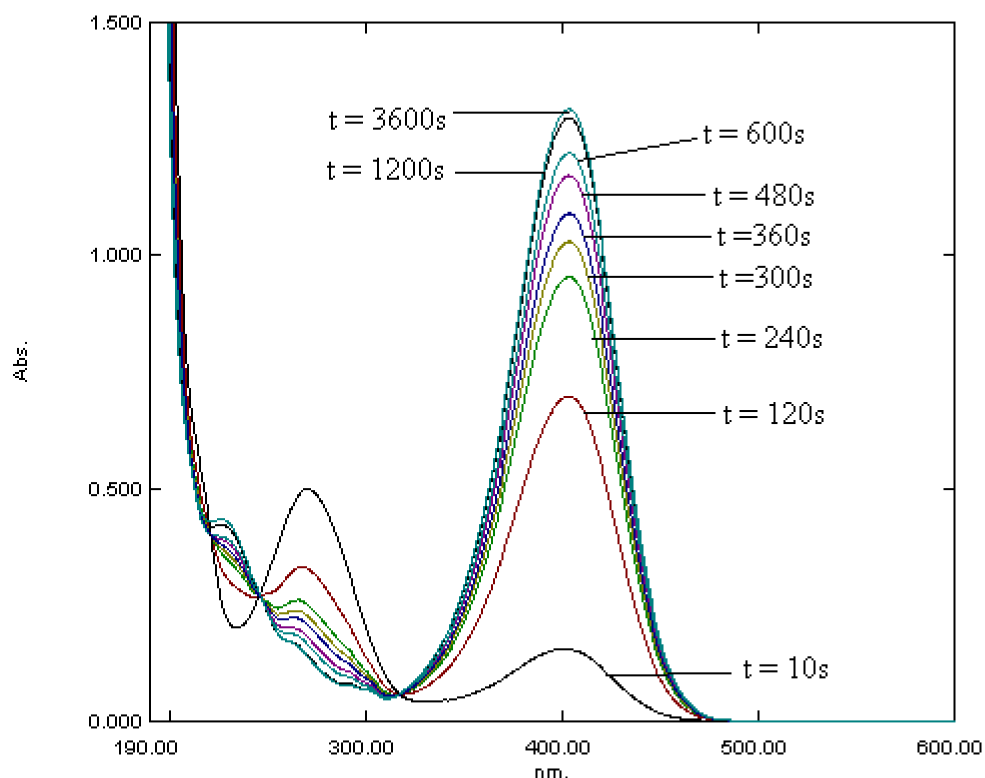


Figure 3-3: UV spectra of alkaline hydrolysis of **26** at 30°C at different reaction time (t) in aqueous solvent containing 6 x 10^{-5} M **26**, 1 x 10^{-3} M NaOH and 80 % CH_3CN.

3.2.1.2 Acidic Hydrolysis of 26 in Mixed Aqueous - Organic Solvents

To determine the suitable wavelength for aqueous hydrolysis of **26** in acidic mixed aqueous-organic solvents, the method described earlier to determine the wavelength in alkaline hydrolysis was used (page 39).

In a typical kinetic run with total volume of 5 ml of reaction mixture containing 0.5 ml of 0.01 M HCl and 4.4 ml H_2O, the reaction mixture was allowed to temperature-equilibrate for ~ 15 minutes at 30 °C. The reaction was then initiated by adding 0.1 ml of 0.003 M of **26** (prepared in CH_3CN) to the temperature-equilibrated reaction mixtures. The resulting aqueous reactions mixture contained 2 % v/v CH_3CN. The UV spectra of reaction mixtures were scanned at different time intervals and are

shown in Fig. 3-4. Similar spectra of reaction mixture at 50 % v/v and 80 % v/v CH_3CN were monitored at different time intervals and are shown in Figs. 3-5 and 3-6.

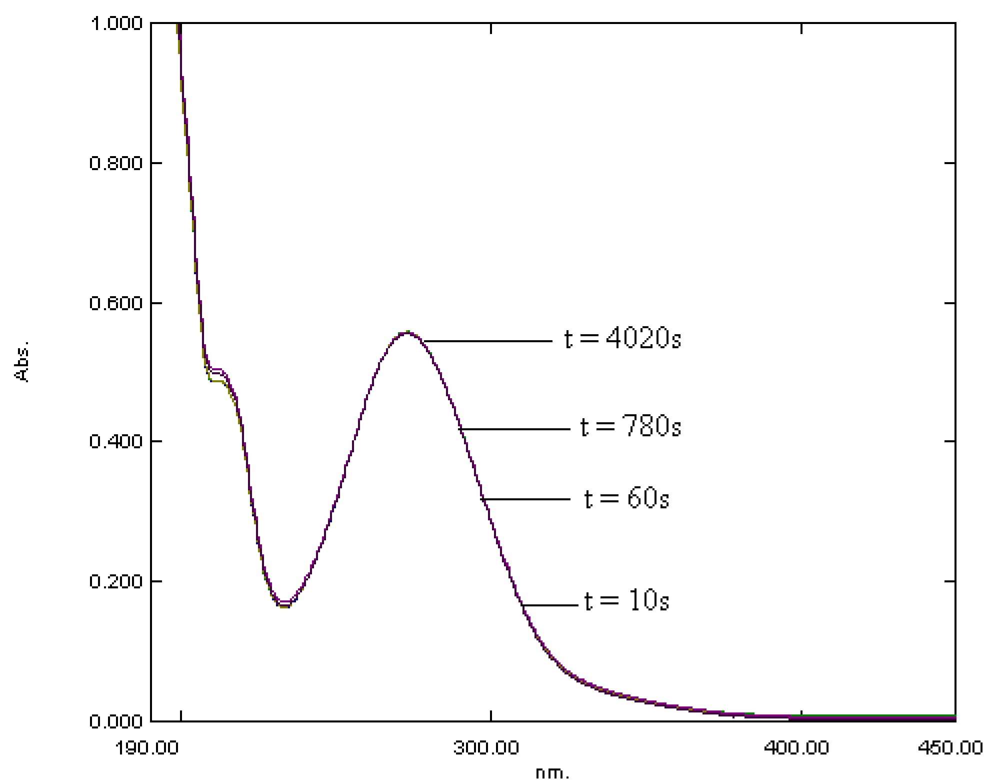


Figure 3-4: UV spectra of acid hydrolysis of **26** at 30°C at different reaction time (t) in aqueous solvent containing 6 x 10^{-5} M **26**, 1 x 10^{-3} M HCl and 2 % CH_3CN.

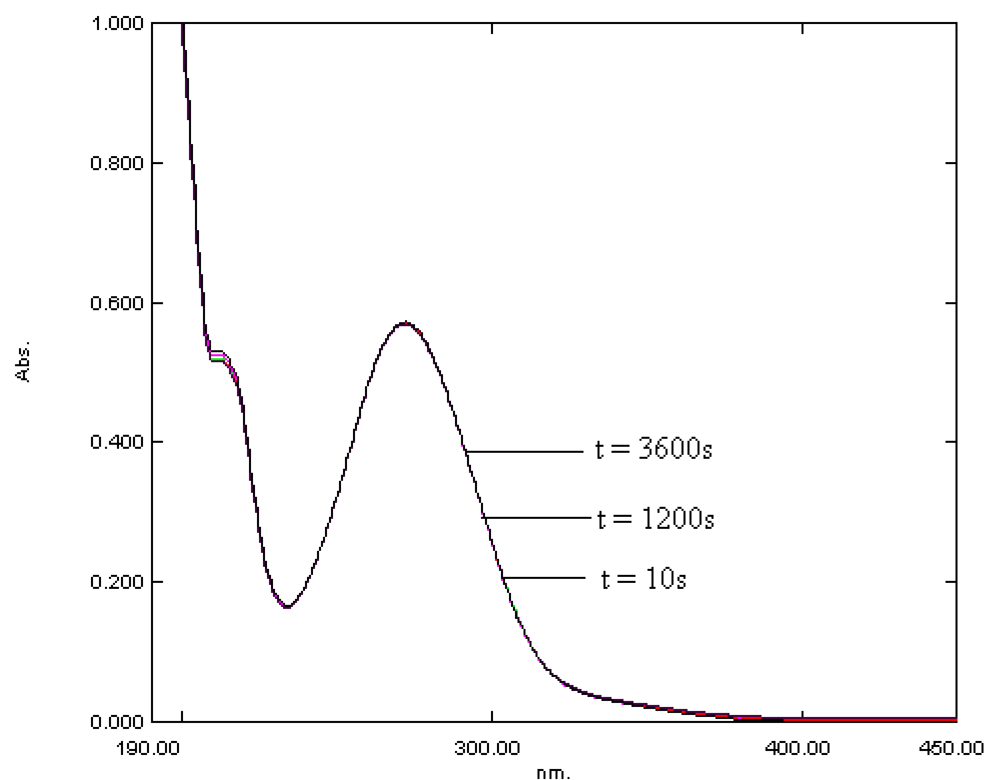


Figure 3-5: UV spectra of acid hydrolysis of **26** at 30°C at different reaction time (t) in aqueous solvent containing 6 x 10^{-5} M **26**, 1 x 10^{-3} M HCl and 50 % CH_3CN.

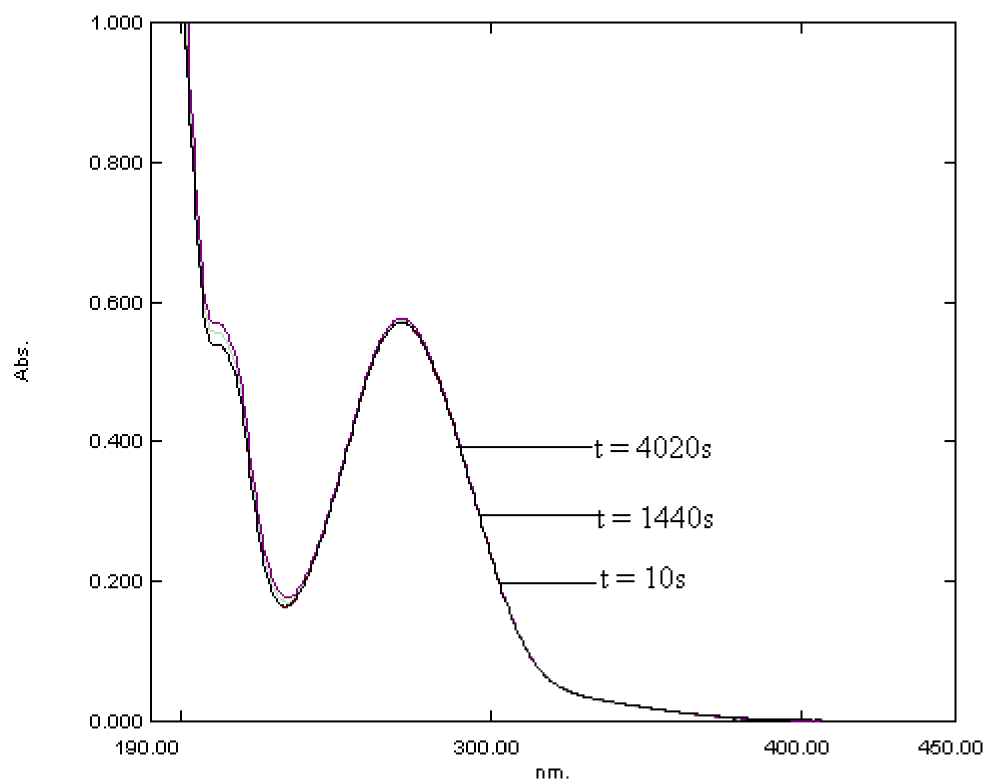


Figure 3-6: UV spectra of acid hydrolysis of **26** at 30°C at different reaction time (t) in aqueous solvent containing 6 x 10^{-5} M **26**, 1 x 10^{-3} M HCl and 80 % CH_3CN.

It is evident from Figs. 3-4 to 3-6, that there are no major different changes in absorbance with the increase of reaction time. As a consequence, the rate of acidic hydrolysis of **26** could not be measured. The alkaline hydrolysis of **26** shows better change in absorbance at 400 nm compare to acidic hydrolysis of **26**. In conclusion, the wavelength at 400 nm was considered for rate measurements.

3.2.2 Experimental Details on Kinetic Measurements

Kinetic measurements were carried out using SHIDMADZU UV-Visible spectrophotometer with the help of UV-1601 PC software and the cell compartment of the spectrophotometer. The temperature of the cell compartment was kept constant at 30 °C. The total volume of reaction mixtures was kept constant at 5 ml. Preparation of kinetic mixture was done shortly before the start of kinetic run. The reaction mixture containing all the reaction component except substrate was put in thermostatic water bath which was set at 30°C for about 15 minutes. The spectrophotometer was standardized with distilled water both as reference and blank sample. The reaction was then initiated by adding an appropriate amount of substrate with micro syringe to the temperature - equilibrated reaction mixture. The reaction mixture was then mixed well and quickly transferred to a glass cuvette which was subsequently placed into the cell compartment of the spectrophotometer. All of these steps from the start of the reaction until the cuvette was placed into the cell compartment took less than 25 seconds.

The reactions were generally carried out for reaction period of more than 6 - 7 halflives. In the case of slow reaction, the sampling method was applied. This technique was applied to all the kinetic runs with observed rate constant, $k_{obs} \leq 1.0 \times 10^{-4}\ s^{-1}$. Measurement of pH was carried out using WITIG digital pH meter, MODEL: W – 500, at room temperature. The pH meter was calibrated with the standard pH

buffer of pH 3.99, 4.01 and 6.99. The standard pH buffer solutions with pH accuracy of ± 0.02 were supplied by Mettler Toledo. The pH was measured before and after each run at 30.0°C. In order to maintain a constant pH, the amines were employed as buffer as well as nucleophile. The amine concentration was maintained in large excess over that of ester in order to obtained pseudo-first-order kinetic condition.

The amines, such as $MeNH_2.HCl$ (**49**), $Me_2NH.HCl$ (**50**), Et_2NH (**37**), $BzNH_2$ (**43**), BzNHMe (**51**), BzNHEt (**52**) and $BzNMe_2$ (**53**), were of reagent grade obtained from Merck, Riedel-de-Haen, Aldrich and Fluka. The stock solution of $MeNH_2.HCl$ (**49**) (1.0 M), $Me_2NH.HCl$ (**50**) (1.0 M), Et_2NH (**37**) (1.0 M), $BzNH_2$ (**43**) (1.0 M), BzNHMe (**51**) (1.0 M), BzNHEt (**52**) (1.0 M) and $BzNMe_2$ (**53**) (1.0 M) were prepared in organic co-solvent (i.e. CH_3CN).

Because of the solubility problem, all the kinetic runs and preparation of stock solutions were carried out in mixed solvent of water - acetonitrile (50 % : 50 % v/v) except for the stock solution of hydrochloric acid which was prepared in water. The amines (buffer) were prepared in double distilled water and acetonitrile HPLC grade supplied by Fischer. The buffers of amine were prepared shortly before the use. Ionic strength of reaction mixture was maintained constant at 0.4 M or 0.3 M by addition of sodium bromide solution. Dilute solutions of substrate ester (**47**) were prepared in double distilled water and acetonitrile (50 % : 50 % v/v).

3.3 Details of Calculation

The K_a values were calculated based on the assumption that at 50 % free base (f_b), the pK_a = pH. This can be shown as follows

$$R\overset{+}{N}H_3 \overset{K_a}{\rightleftharpoons} RNH_2 + H^+ \quad (3 - 1)$$

$$pK_a = pH + \log([RNH_3^+])/([RNH_2]) \quad (3-2)$$

At $f_b = 0.5$, $[RNH_3^+] = [RNH_2]$ and hence Eq. (3-2) leads to

$$pK_a = pH \quad (3-3)$$

The values of f_b were calculated based on the Eq. (3-4)

$$f_b = K_a/(a_H + K_a) \quad (3-4)$$

3.3.1 Simple First - Order Rate Constant

Kinetic studies were performed under pseudo-first order reaction conditions. The reaction, under such conditions may be expressed as

$$A \xrightarrow{k_{obs}} P \quad (3 - 5)$$

In view of Eq. (3-5), the rate of reaction may be expressed by rate law

$$Rate = -(d[A]/dt) = k_{obs}[A] \quad (3-6)$$

Integration of Eq. (3-6) gives

$$[A] = [A_0] \exp(-k_{obs} t) \quad (3-7)$$

where $[A_0]$ is the initial concentration of A and [A] is the concentration of A at any reaction time. If A_{obs} is the observed absorbance of the reaction following Eq. (3-8) then

$$A_{obs} = \delta_A [A] + \delta_p [P] \quad (3\text{-}8)$$

where δ represent molar extinction coefficient of a particular species. From Eqs. (3-5) and (3-6), it can be shown that

$$[A_0] = [A] + [P] \quad (3\text{-}9)$$

Eqs. (3-8) and (3-9), give

$$A_{obs} = \delta_A [A] + \delta_p ([A_0] - [A])$$

$$= (\delta_A - \delta_p)[A] + \delta_p [A_0] \quad (3\text{-}10)$$

If $\delta_P [A_0] = A_\infty$ and $(\delta_A - \delta_P) = \delta_{app}$, Eq. (3-10) can be simplified to

$$A_{obs} = \delta_{app} [A] + A_\infty \quad (3\text{-}11)$$

Substitution of Eq. (3-7) into (3-11) and replacing $[A_0] = [X_0]$, one gets

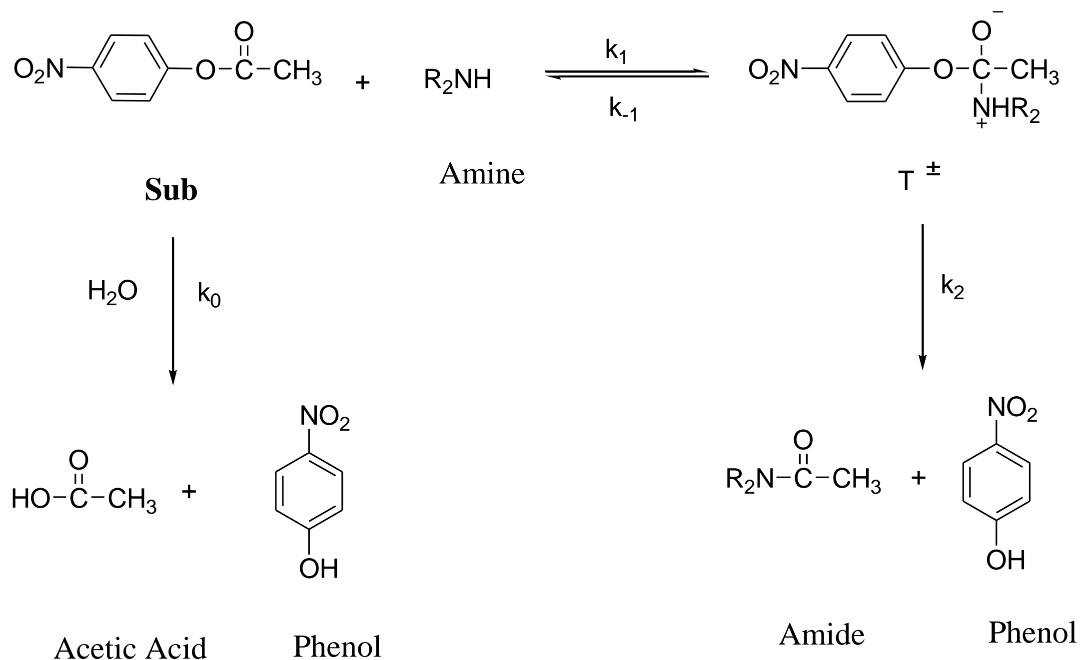
$$A_{obs} = \delta_{app} [X_0] \exp(- k_{obs} t) + A_\infty \quad (3\text{-}12)$$

The observed data (A_{obs} versus reaction time, t) were found to fit to Eq. (3-12) where δ_{app} is molar extinction coefficient of the reaction mixture, A_∞ is absorbance at the reaction time, $t = \infty$, k_{obs} is pseudo-first-order rate constant and $[X_0]$ represents the initial concentration of **26**. Eq. (3-12) is applied to reaction conditions where the disappearance of reactant is monitored as a function of reaction time, t. When the appearance of product is monitored as a function of t, the equation will be converted to Eq. (3-13) as follow:

$$A_{obs} = \delta_{app} [X_0] [1- \exp(- k_{obs} t)] + A_0 \quad (3\text{-}13)$$

The observed data (A_{obs} versus t), obtained for all kinetic runs obeyed Eq. (3-13).

3.3.2 Rate Law for Aminolysis of 26



Scheme 3-1

3.3.3 The Observed Rate Law

$$\text{rate} = k_{obs} [\text{Sub}] \quad (3\text{-}14)$$

where

$$k_{obs} = k_0 + k_b [RNH_2] \quad (3\text{-}15)$$

The derived rate law:

$$\text{rate} = k_2 [T] \quad (3\text{-}16)$$

$$d[T]/dt = k_1[Sub][RNH_2] - k_{-1}[T] - k_2[T] \qquad (3\text{-}17)$$

Since T is short lived highly reactive tetrahedral intermediate, therefore applying the steady state approximation to the rate of change of [T], one gets

$$0 = k_1[Sub][RNH_2] - [k_{-1} + k_2][T] \qquad (3\text{-}18)$$

$$[T] = (k_1[Sub][RNH_2]) / (k_{-1} + k_2) \qquad (3\text{-}19)$$

from Eqs. (3-18) and (3-19) we get the derived rate law

$$\text{rate} = [(k_1 k_2)/([k_{-1} + k_2])] [RNH_2] \qquad (3\text{-}20)$$

comparing Eq. (3.19) and Eq. (3-20), we get Eq. (3-21)

$$k_{obs} = [(k_1 k_2)/(k_{-1} + k_2)] [RNH_2] \qquad (3\text{-}21)$$

where

$$k_n = (k_1 k_2)/(k_{-1} + k_2) \qquad (3\text{-}22)$$

3.4 Product Characterizations

The product characterization studies were carried out using RP-HPLC. The term “reversed phase” implies the use of a polar eluent with a polar molecules. In this case water can be used as an eluent. This is an important in order to reduce the usages of organic solvent beside reduce cost and it is more environmental friendly.

The standard methods were determined by several trial and error. The most important aspect using this method is to determine the most suitable mobile phase in order to get the best separation.

3.4.1 Experimental Details

3.4.1.1 Reverse-Phase High Performance Liquid Chromatography (RP-HPLC)

The HPLC system was composed of LC-20AT Prominence pumps, SPD-20A Prominence UV/Vis detector, DGU-20A5 prominence degasser, 7725i-VS manual system Injector and CBM-20A System Controller (all from Shidmadzu, Kyoto, Japan, except Injector from Rheodyne, USA). The chromatographic and the integrated data were recorded using a Dell computer system. The chromatographic columns used in the present work are ODS Hypersil C_{18} (250 mm x 4.6 mm) size 5 µm (Thermo Electron Corporation, Runcom, UK).

3.4.1.2 Materials

Benzylamine (**43**) was purchased from Riedel-de-Haen. *N*,*N*-diethylamine (**37**) and *p*-nitrophenol (**29**) were purchased from Aldrich. *N*,*N*-Dimethylbenzylamine (**53**) and *p*-nitrophenyl acetate (**26**) were purchased from Fluka. *N*-Benzylacetamide

(**44**), *N*,*N*-diethylacetamide (**45**), *N*,*N*-(diethylaminomethyl)benzyl alcohol (**35**), 2-((diethylamino)methyl)benzyl acetate (**42**) were synthesis using reported method. The HPLC grade acetonitrile, 2-propanol was purchased from Fischer Scientific. Double distilled water was used in all experimental preparations.

3.4.1.3 Standard Solutions

N-Benzylacetamide (**44**), *N*,*N*-diethylacetamide (**45**), *N*,*N*-(diethylaminomethyl)benzyl alcohol (**35**), 2-((diethylamino)methyl)benzyl acetate (**42**), *p*-nitrophenyl acetate (**26**) and phenolate ion (**48**) were accurately weight and dissolved in the mixed solvents of 50 % MeCN : 50 % H_2O with concentration 0.001 M. Benzylamine, *N*,*N*-diethylamine, *N*,*N*-dimethylbenzylamine and *N*,*N*-(diethylaminomethyl)benzyl alcohol were prepared at concentration of 0.05 M, 0.2 M, 0.1 M and 0.08 M respectively with total ionic strength = 0.4 M in CH_3CN : 50 % v/v. For the determination of retention times, the reference standards were injected both individually and as a mixture.

3.4.1.4 Chromatographic Conditions

The analysis was carried out using ODS Hypersil C_{18} (250mm x 4.6mm i.d.; particle size 5 µm) column using as stationary phase. The mobile phase was H_2O:2-PrOH (80 %: 20 % v/v) for secondary and tertiary amine and (65 %: 35 % v/v) for amino alcohol and primary amine. Isocratic conditions were maintained for the whole experiment. The flow rate was kept constant at 1.0 ml/min and column was maintained at room temperature. The injection volume was 20 µl and the detection was performed at 254 nm using UV-Vis detector. The temperature was set at room temperature.

3.4.1.5 Determination of Mobile Phase

The suitable mobile phase was tested by the combination of different solvents. The combination of water and organic solvent was determined by trial-and-error based on the separation of standard compounds. The best results were obtained with the combination of H_2O and 2-PrOH in ratio of 65 % H_2O: 35 % 2-PrOH (mobile phase A) for primary amine and amino alcohol and 80 % H_2O: 20 % 2-PrOH (mobile phase B) for secondary and tertiary amines.

3.4.1.6 Sample Preparations

Preparation of samples was based on the samples preparation for kinetic runs. The same conditions were applied. The amine concentration was maintained in large excess over that of ester in order to obtain pseudo-first-order kinetic. Compare to sample for kinetic run, the concentration of substrate (**26**) for HPLC analysis was increase from 1 x 10^{-6} M to 0.001 M in order to determine clear peaks in HPLC chromatogram. The total buffer was set at 50 % free base and ionic strength was maintained at 0.4 M by addition of sodium bromide. In a typical sample with total volume of 5 ml of reaction mixture which contained buffer, benzylamine (0.05 M), *N,N*-diethylamine (0.2 M), *N,N*-dimethylbenzylamine (0.1 M) and *N,N*-(diethylaminomethyl)benzyl alcohol (0.08 M) was prepared. The reaction mixture was allowed to temperature-equilibrate for ~ 15 minutes at 30 °C. The reaction was then initiated by adding 0.089 ml of 0.056 M of **26** to the temperature-equilibrated reaction mixtures. The characterization of product was made by monitoring the appearance and disappearance of peaks at different time during the reaction occurred. An aliquot of 20 μl of reaction mixtures were injected manually through injector at different fraction of time for HPLC analysis.

3.4.2 Results and Discussions

3.4.2.1 Primary Amine

In general, observations can be made by monitoring the increase and decrease of peaks representing starting material (**26**) and products. The identification of product was made by comparing the retention time and the peak area of reaction mixture with standard. Fig. 3-7 and Table 3-1 show the detail data for standard compounds.

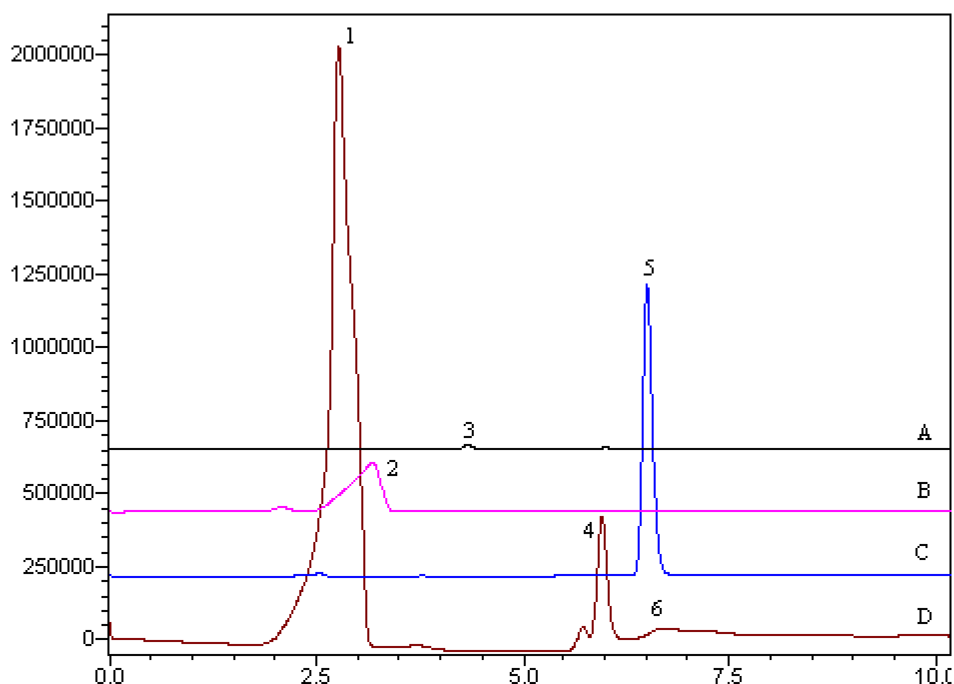


Figure 3-7: HPLC chromatogram: 1 = NaBr, μ = 0.4 M, 2 = **48** (0.001 M), 3 = **44** (0.001 M), 4 = **43** (0.10 M) and 5 = **26** (0.001 M). Eluent : Isopropanol/water (35 % : 65 % v/v), column temperature = room temperature, injected volume = 20 μl.

Table 3-1: The Summary of the Data of Standard Compounds for Fig. 3-7

Chromatogram	Peaks	Compounds	Ret. Time (min)	10^{-4} Area	10^{-3} Height
A	3	**44**	4.312	12	16
B	2	**48**	3.173	442	168
C	5	**26**	6.512	855	996
D	1	NaBr	2.773	4394	2059
	4	**43**	5.963	457	465
	6	-	-	-	-

Fig. 3-8 and Table 3-2 show the details of the data of reaction mixture. In Fig. 3-8, chromatogram A showed the peak of reaction mixture at initial time. Peak 5 represents substrate **26** in highest concentration. Chromatogram B was run at t = ∞ (refer to the time taken to complete the reaction based on the sample in kinetic run - Chapter 4). Here we can see the disappearance of peak 5 and the appearance of products, peak 2 and peak 3 which represent **48** and **44** respectively. This result was in agreement with the early hypothesis that, aminolysis of ester by primary amine will give an amide as a product. The detailed proposed mechanism was shown in Scheme 3-1.

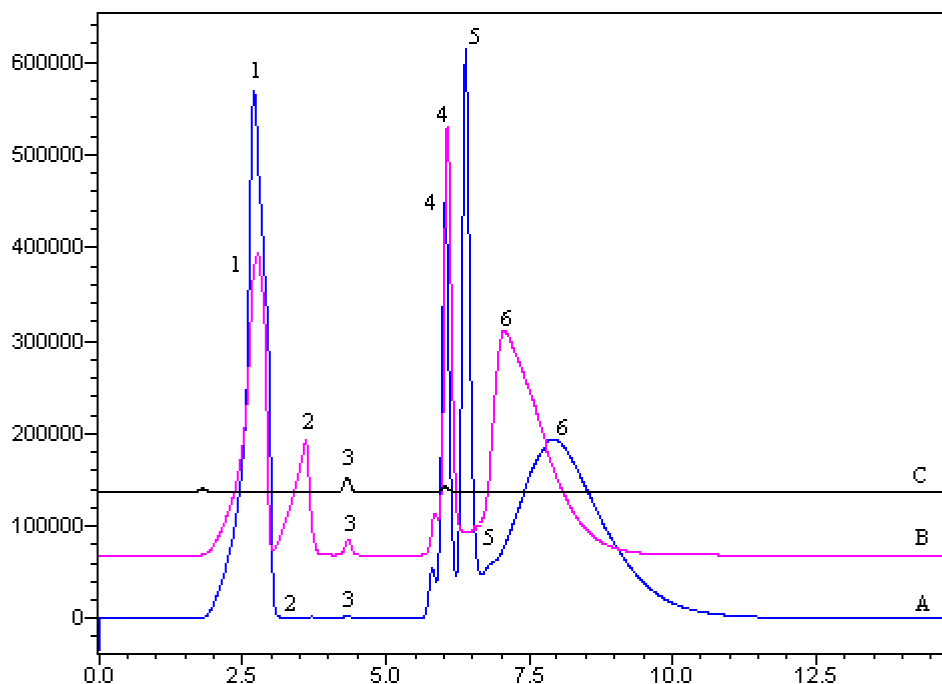


Figure 3-8: HPLC chromatogram shows the peaks of reaction mixture at, t = 0 (A) and t = ∞ (B). C is the chromatogram of standard product, **44**. Eluent : Isopropanol/water (35 % : 65 % v/v), column temperature = room temperature, injected volume = 20 µl.

Table 3-2: The Summary of the Data of Reaction Mixture for Fig. 3-8.

Chromatogram	Peaks	Compounds	Ret. Time (min)	10^{-4} Area	10^{-3} Height
A	1	NaBr	2.699	1396	568
	2	**48**	-	-	-
	3	**44**	4.314	3	4
	4	**43**	6.019	431	464
	5	**26**	6.374	642	618
	6	-	-	-	-
B	1	NaBr	2.762	814	325
	2	**48**	3.597	253	126
	3	**44**	4.329	15	18
	4	**43**	6.058	453	466
	5	**26**	-	-	-
	6	-	-	-	-
C	3	**44**	4.312	12	16

3.4.2.2 Secondary Amine

The identification of product was carried out by comparing the retention time and the peak area of reaction mixture with standard. Fig. 3-9 and Table 3-3 show the details of the data for standard compounds. Fig. 3-10 and Table 3-4 show the details of the data of reaction mixture. In Fig. 3-10, chromatogram A shows the peaks of reaction mixture at initial time. Peak 4 represents substrate **26** in highest concentration. Chromatogram B was run at t = ∞ (refer to the time taken to complete the reaction based on the sample in kinetic run - Chapter 4). Here we can see the disappearance of peak 4 and the appearance of suspected products, peak 2 and peak 5 which represent **48** and **45** respectively. This result was in agreement with the early hypothesis that, aminolysis of ester by secondary amine will give an amide as a product.

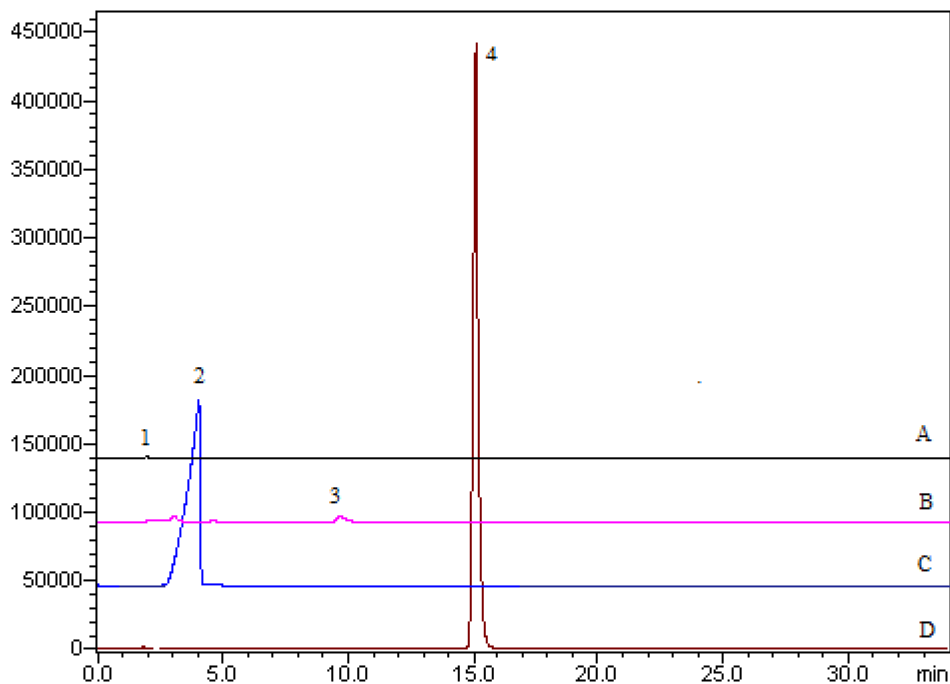


Figure 3-9: HPLC chromatogram: 1 = **45** (0.001M), 2 = **48** (0.001 M), 3 = **37** (0.2 M) and 4 = **26** (0.001 M). Eluent: Isopropanol/water (20 % : 80 % v/v), column temperature = room temperature, injected volume = 20 μl.

Table 3-3: The Summary of the Data of Standard Compounds for Fig. 3-9

Chromatogram	Peaks	Compounds	Ret. Time (min)	10^{-3} Area	10^{-2} Height
A	1	**45**	1.948	15	20
B	2	**48**	4.044	4708	1348
C	3	**37**	9.666	106	43
D	4	**26**	15.078	8161	4419

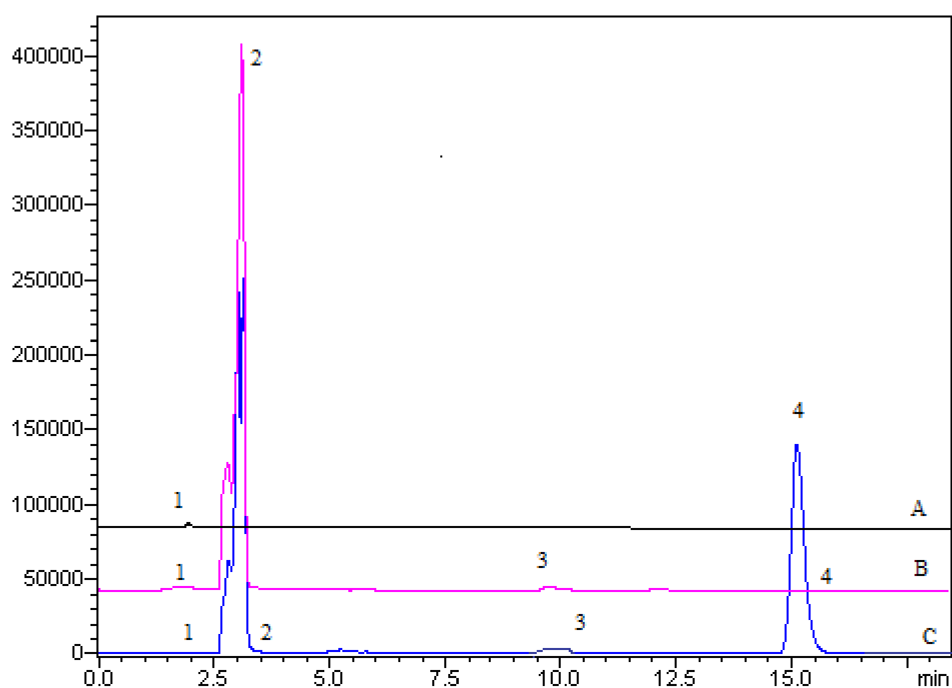


Figure 3-10: HPLC chromatogram shows the peaks of reaction mixture at, t = 0 (C) and t = ∞ (B). A is the chromatogram of standard product, **45**. Eluent: Isopropanol/water (20 % : 80 % v/v), column temperature = room temperature, injected volume = 20 µl.

Table 3-4: The Summary of the Data of Reaction Mixture for Fig. 3-10.

Chromatogram	Peaks	Compounds	Ret. Time (min)	10^{-3} Area	10^{-2} Height
A	1	**45**	1.948	15	20
B	1	**45**	-	-	-
	2	**48**	3.005,3.128	3373	2565
	3	**37**	9.781	5	3
	4	**26**	15.118	2783	1401
C	1	**45**	1.975	38	17
	2	**48**	3.096	4117	3644
	3	**37**	9.762	53	24
	4	**26**	-	-	-

3.4.2.3 Tertiary Amine

The product characterization was carried out by comparing the retention time and the peak area of reaction mixture with standard. Fig. 3-11 and Table 3-5 show the details of the data for standard compounds.

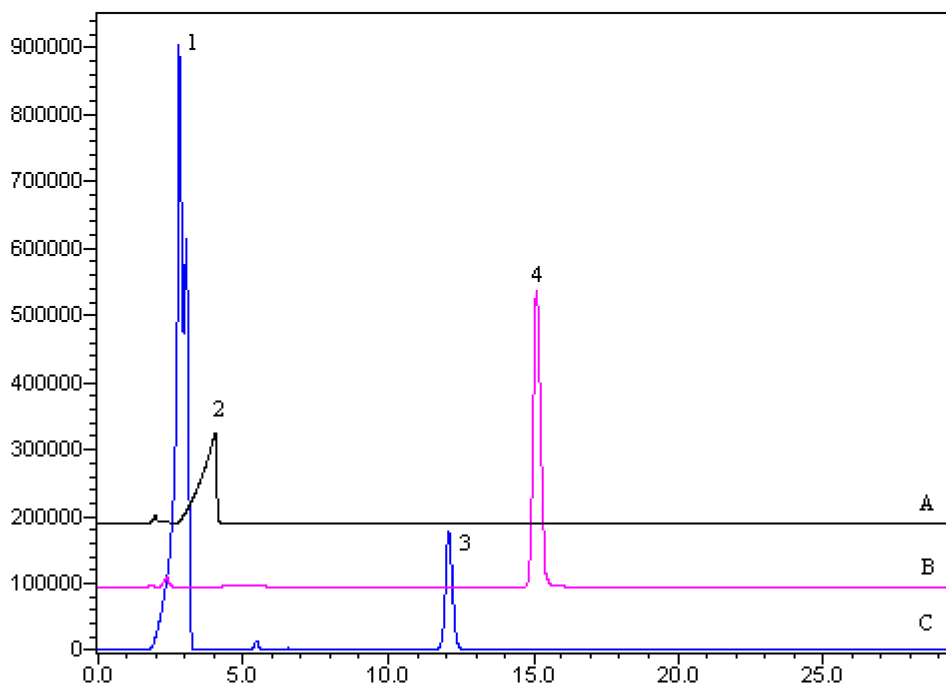


Figure 3-11: HPLC chromatogram: 1 = NaBr, μ = 0.4 M, 2 = **48** (0.001 M), 3 = **53** (0.10 M), 4 = **26** (0.001 M). Eluent: Isopropanol : water (20 % : 80 % v/v), column temperature = room temperature, injected volume = 20 μl.

Table 3-5: The Summary of the Data of Standard Compounds for Fig. 3-11.

Chromatogram	Peaks	Compounds	Ret. Time (min)	10^{-4} Area	10^{-3} Height
A	1	NaBR	2.805,3.054	2157	905
	3	**53**	12.075	291	178
B	4	**26**	15.078	816	442
C	2	**48**	4.044	471	135

Fig. 3-12 and Table 3-6 show the details of the data of reaction mixture. In Fig. 3-12, chromatogram A shows the peaks of reaction mixture at initial time. Peak 4 represent substrate **26** in highest concentration. Chromatogram B was run at t = ∞. Here we can see the disappearance of peak 4 and the appearance of suspected product, peak 2 which represent **48**. This result was in agreement with the hypothesis that, hydrolysis of ester by tertiary amine will gave **48** as a final product. The detail proposed mechanism was showed in Scheme 3-1.

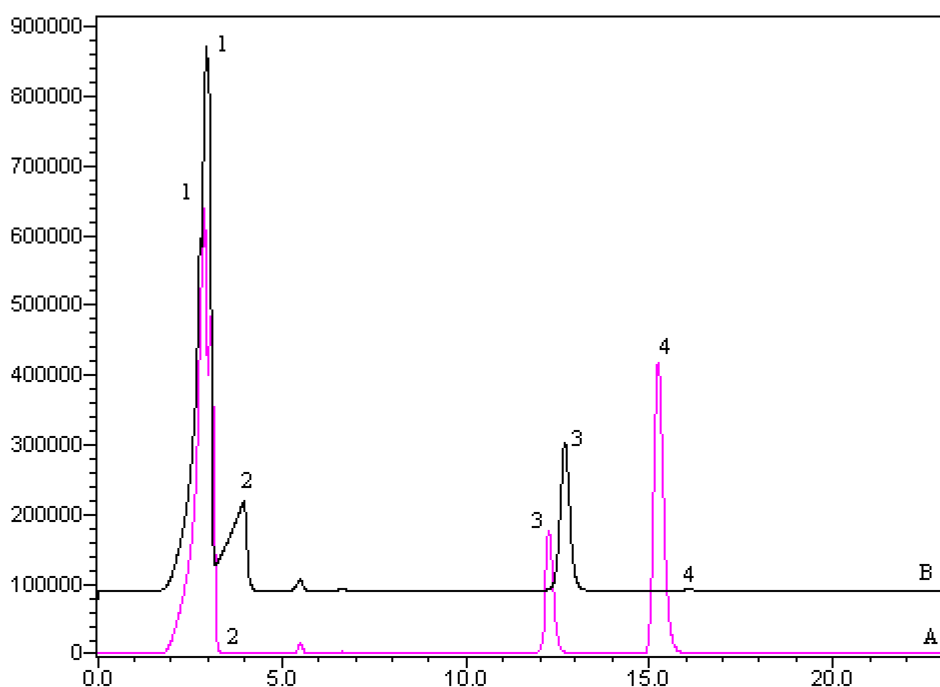


Figure 3-12: HPLC chromatogram shows the peaks of reaction mixture at, t = 0 (A) and t = ∞ (B). Eluent: Isopropanol : water (20 % : 80 % v/v), column temperature = room temperature, injected volume = 20 µl.

Table 3-6: The Summary of the Data of Reaction Mixture for Fig. 3-12.

Chromatogram	Peaks	Compounds	Ret. Time (min)	10^{-4} Area	10^{-3} Height
A	1	NaBr	2.906,3.078	1828	640
	2	**48**	-	-	-
	3	**53**	12.282	299	176
	4	**26**	15.247	791	419
B	1	NaBr	2.783,2.975	1981	782
	2	**48**	3.964	441	128
	3	**53**	12.716	400	212
	4	**26**	16.068	6	3

3.4.2.4 Amino Alcohol

The product characterization was carried out by comparing the retention time and the peak area of reaction mixture with the retention time and peak area of standard compounds. Fig. 3-13 and Table 3-7 show the detailed data for standard compounds. Fig. 3-14 and Table 3-8 show the detailed data of reaction mixture. In Fig. 3-14, chromatogram A showed the peaks of reaction mixture at initial time. Peak 5 represent substrate **26** in highest concentration. Chromatogram B was run at $t = \infty$. Here we can see the disappearance of peak 5 and the appearance of suspected product, peak 2 which represent **48**.

The suspected product was not detected in chromatogram. In order to affirm that there is no formation of intermediate (**42**), 0.1 ml of 0.001 M ester was mix with 0.1 ml of reaction mixture and final mixture was run at $t = t_{1/2}$ as shown in Fig. 3-15. We can see an extra peak 6 in chromatogram B compare with pure reaction mixture represented by chromatogram A. The same was applied for $t = \infty$ (reaction finished) as shown in Fig. 3-16. This result was not as we expected, due to the higher values of k_{obs} for aminolysis of **26** by **35** compared to the values of k_{obs} for aminolysis of **26** by tertiary amine. We expected that aminolysis of **26** by **35** occurred through direct attack

of nucleophilic hydroxyl group which gave **42** as an intermediate (peak 6). Based on the result from HPLC we can conclude that, the reaction of **26** with **35** occurred through direct attack of the nucleophilic amine group.

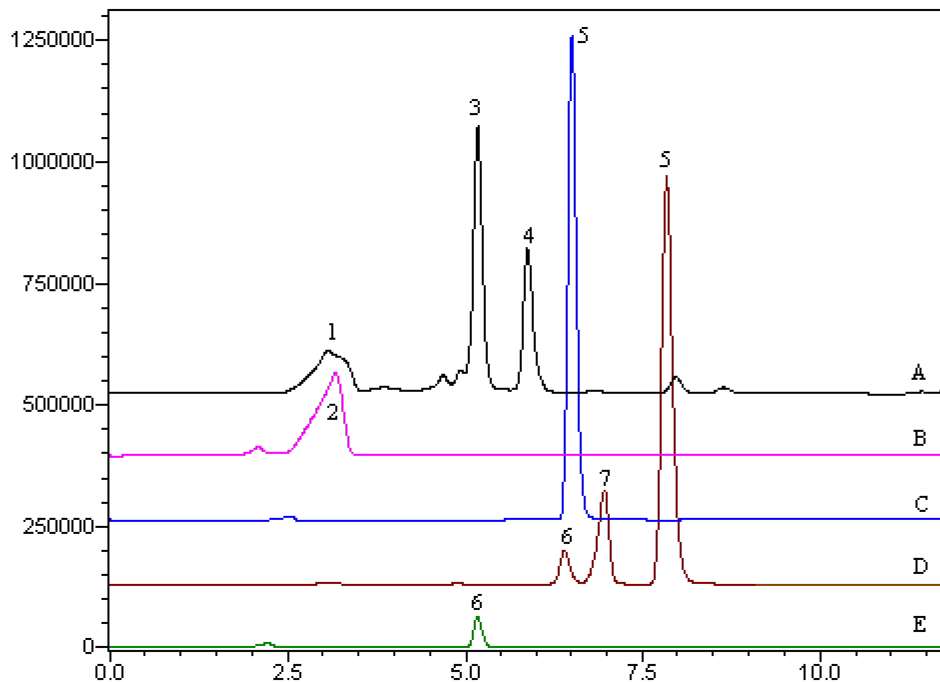


Figure 3-13: HPLC chromatogram: 1 = NaBr, μ = 0.4 M, 2 = **48** (0.001 M), 3 & 4 = **35** (0.08 M), 5 = **26** (0.001 M), 6 = **42** (0.001 M) and 7 = **29** (0.001 M). Eluent: Isopropanol : water (35 % : 65 % v/v), column temperature = room temperature, injected volume = 20 μl.

Table 3-7: The Summary of the Data of Standard Compounds for Fig. 3-13.

Chromatogram	Peaks	Compounds	Ret. Time (min)	10^{-4} Area	10^{-3} Height
A	1	NaBr	3.064	297	90
	3	**35**	5.162	515	552
	4	**35**	5.863	327	298
B	2	**48**	3.173	442	168
C	5	**26**	6.512	855	996
D	5	**26**	7.843	921	839
	6	**42**	6.408	72	70
	7	**29**	6.973	218	195
E	6	**42**	5.161	57	65

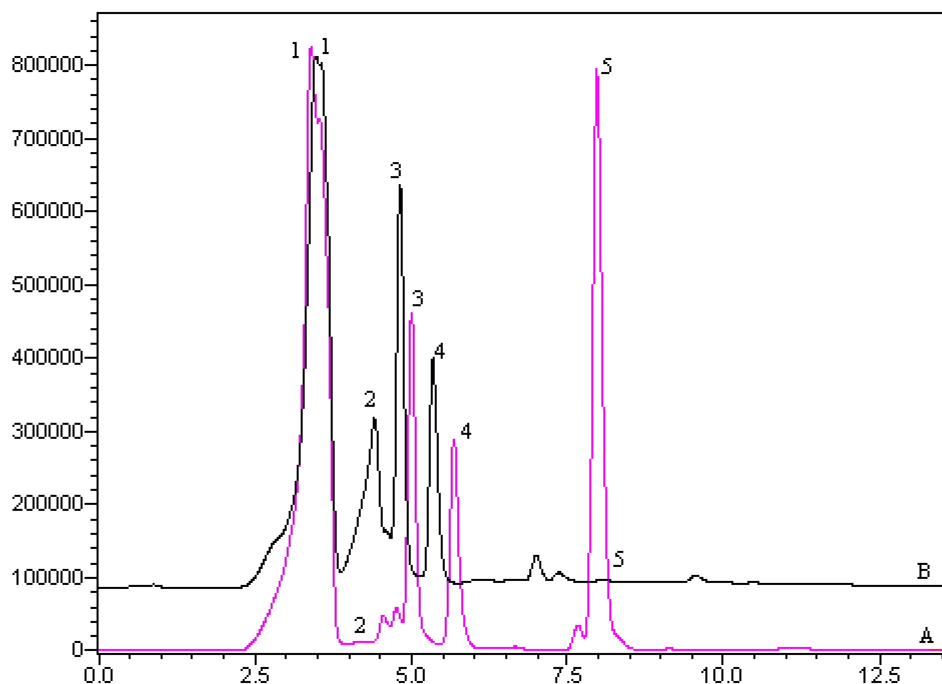


Figure 3-14: HPLC chromatogram shows the peaks of reaction mixture at, t = 0 (A) and t = ∞ (B) which represented by A and B respectively. Eluent: Isopropanol : water (35 % : 65 % v/v), column temperature = room temperature, injected volume = 20 µl.

Table 3-8: The Summary of the Data of Reaction Mixture for Fig. 3-14.

Chromatogram	Peaks	Compounds	Ret. Time (min)	10^{-4} Area	10^{-3} Height
A	1	NaBr	3.393, 3.527	2367	827
	2	**48**	4.149	22	13
	3	**35**	4.994	427	464
	4	**35**	5.667	298	290
	5	**26**	7.981	911	795
	6	**42**	-	-	-
B	1	NaBr	3.469, 3.553	2006	729
	2	**48**	4.405	506	231
	3	**35**	4.812	488	549
	4	**35**	5.338	306	313
	5	**26**	-	-	-
	6	**42**	-	-	-

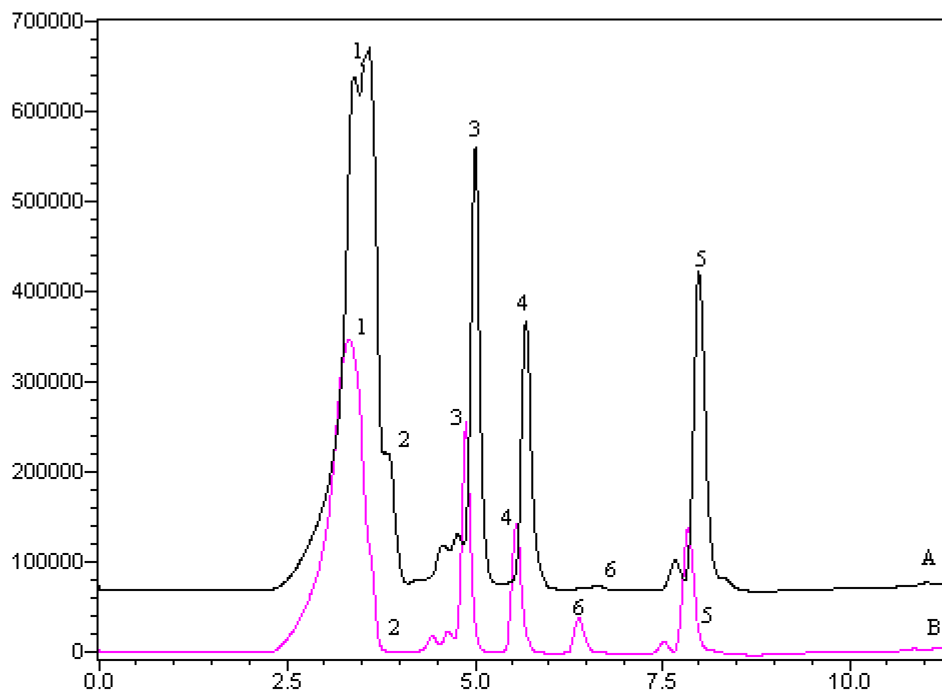


Figure 3-15: HPLC chromatogram shows the peaks of reaction mixture at, t = 4260 s (~ $t_{1/2}$). Chromatogram A shows pure reaction mixtures while chromatogram B shows the mixture of 0.1 ml of **42** (0.001 M) and 0.1 ml reaction mixture. Eluent : Isopropanol : water (35 % : 65 % v/v), column temperature = room temperature, injected volume = 20 μl.

Table 3-9: The Summary of the Data of Reaction Mixture for Fig. 3-15.

Chromatogram	Peaks	Compounds	Ret. Time (min)	10^{-4} Area	10^{-3} Height
A	1	NaBr	3.397, 3.574	2127	602
	2	**48**	-	-	-
	3	**35**	4.993	436	491
	4	**35**	5.669	300	299
	5	**26**	7.995	409	353
	6	**42**	-	-	-
B	1	NaBr	3.320	1160	350
	2	**48**	-	-	-
	3	**35**	4.872	218	259
	4	**35**	5.540	139	146
	5	**26**	7.849	165	143
	6	**42**	6.398	46	42

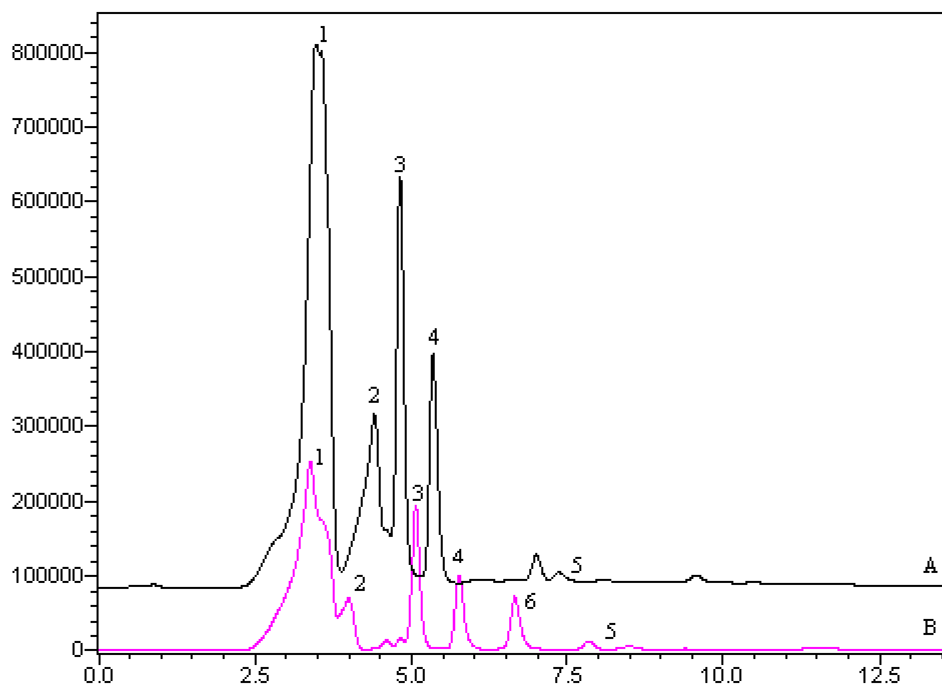


Figure 3-16: HPLC chromatogram shows the peaks of reaction mixture at, t = ∞. Chromatogram A shows pure reaction mixtures while chromatogram B shows the mixture of 0.1 ml of **73** (0.001 M) and 0.1 ml reaction mixture. Eluent: Isopropanol : water (35 % : 65 % v/v), column temperature = room temperature, injected volume = 20 μl.

Table 3-10: The Summary of the Data of Reaction Mixture for Fig. 3-16.

Chromatogram	Peaks	Compounds	Ret. Time (min)	10^{-4} Area	10^{-3} Height
A	1	NaBr	3.469, 3.553	2006	729
	2	**48**	4.405	506	231
	3	**35**	4.812	488	549
	4	**35**	5.338	306	313
	5	**26**	-	-	-
	6	**42**	-	-	-
B	1	NaBr	3.880	867	253
	2	**48**	3.996	99	70
	3	**35**	5.061	176	196
	4	**35**	5.746	102	100
	5	**26**	-	-	-
	6	**42**	6.639	78	70

3.5 References

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