5. MONITORING OF THE AMINOLYSIS REACTIONS -
A SPECTROPHOTOMETRIC INVESTIGATION

5.1 Introduction

From the preceding chapters 3 & 4 it is clear that the esterification reactions of the acyl benzo(thiazoline/oxazoline)-2-thione are sluggish or rather needs photochemical conditions; however the aminolysis reactions are very fast and it require only 10-20 minutes for completion. Although, 3-acyl benzothiazoline-2-thione (4) and 3-acyl benzoxazoline-2-thione (5) have received attention as activated carboxyl compounds, no study which monitors the aminolysis reaction at an activated carboxyl component has been carried out. For a systematic investigation of the course of the aminolysis reactions, a thorough understanding of the electronic absorption spectra of the compounds are inevitable. The nature of the ultraviolet-visible spectrum of 2-mercaptobenzothiazole (1) and 2-mercaptobenzoxazole (2) and their derivatized analogues under different conditions are considered here. However, no attempt has been made for a quantitative evaluation of the kinetic parameters involved in the reactions due to the very fast nature of the process.
Therefore, the present study is envisaged to scan the UV-visible absorption spectrum of 3-acyl benzothiazoline-2-thione (4) and 3-acyl benoxazoline-2-thione (5) during aminolysis and to correlate these with the extent of the reaction. These correlations are made by observing the intensity of the absorption bands at specified wavelengths under different experimental conditions.

5.2 Results and Discussion

5.2.1 UV-visible spectrum of 3-acyl benzothiazoline-2-thione

The two absorption bands $\lambda_1$ and $\lambda_2$ in the UV-visible spectrum of 2-mercaptobenzothiazole (1) in chloroform are found to be at 328 and 224 nm (Fig. 5.1). The observed absorbance of these bands being 1.64 and 0.27 respectively. These bands can be explained from the transition occurring in the tautomerised thiocarbonyl chromophore. When these thiol is derivatized with acids and the UV-visible spectrum taken at the same dilution shows that $\lambda_1$ band at 328 nm is shifted to a shorter wavelength (296 nm) together with considerable decrease in the intensity of absorption (1.32, Fig. 5.2). This can be very well explained on the basis of the thioester formation.
Fig. 5.1 UV-visible spectrum of 2-mercaptobenzothiazole

Fig. 5.2 UV-visible spectrum of 3-benzoyl benzothiazoline-2-thione
5.2.2 UV-visible spectrum of 3-acyl benzothiazoline-2-thione during aminolysis

Acyl derivatives of 2-mercaptobenzothiazole on treatment with amines result into the regeneration of 2-mercaptobenzothiazole. This is evident from the spectrum obtained during the reaction, in which the characteristic band at 328 is regenerated and at the same time the intensity increases. This phenomenon is successfully utilised in the spectrophotometric monitoring of the aminolysis reaction.

Thus, a very dilute solution of 3-benzoyl benzothiazoline-2-thione in chloroform was mixed with an equimolar amount of aniline in chloroform. Stirred well, a definite quantity of the mixture was withdrawn at regular intervals of time (1 min) and absorbance was measured using a spectrophotometer. It was observed that as the reaction proceeds, the intensity of the band, at 328 nm increases gradually and reaches a limiting value (Fig. 5.3). The band at 328 nm is due to the regeneration of 2-mercaptobenzothiazole during the reaction. This regular increase in intensity of the band is considered as the direct measure of the extent of the reaction and hence spectrophotometric monitoring is possible.
In order to make a quantitative correlation the absorbance was studied at the wavelength of maximum absorption (viz: 328 nm). The mixture was introduced into the spectrophotometer and the absorbance was measured at regular intervals of time until constant values were obtained. Absorbance at infinite time (1 h) was also taken. A graph was plotted by taking relative absorbance

\[ D_{rel} = \frac{D_t - D^0}{D^\infty - D^0} \]

(where \(D_t\) - is the absorbance at a specific time, \(D^0\) - at the initial time and \(D^\infty\) is the absorbance at infinite time) on Y axis and time on X-axis.

Fig. 5.3 UV-visible spectrum of 3-acyl benzothiazoline-2-thione during aminolysis
Fig. 5.4 is a plot of the reaction between 3-benzoyl benzothiazoline-2-thione (4a) and aniline. The smooth reaction reaches 90% completion within ten minutes and after that it is very slow. The above spectrophotometric monitoring was extended to other amines such as benzylamine, amino alcohols and aminophenols (Fig. 5.5). From the plot, it is clear that 3-benzoyl benzothiazoline-2-thione reacts with o-aminophenol very quickly and the reaction is around 70% at the very first minute itself. However, the reaction with ethanolamine is very slow. The reaction is very slow initially and it requires around 20 minutes for 80% completion. The behaviour of benzylamine is almost similar to that of aniline.

Fig. 5.4 Reaction of 3-benzoyl benzothiazoline-2-thione with aniline
Analogous monitoring studies were also carried out with 3-(phenylacetyl) benzothiazoline-2-thione (4b). Fig 5.6 is the plot obtained with relative absorbance against time in minutes. Similar to 3-benzoyl benzothiazoline-2-thione, the reaction with aniline is very fast. At the first minute itself the reaction reaches to 60% completion and after that it gradually attains the limiting value. However, the reaction between o-aminophenol and compound (4b) is very sluggish. The reaction is very slow initially, but
gradually the rate increases. The behaviour of ethanolamine with 4b is almost similar to that of 3-benzoyl benzothiazoline-2-thione (4a). The reaction is slow throughout.

![Fig. 5.6 Reaction of 3-(phenylacetyl) benzothiazolined-2-thione with amines](image)

The general nature of this monitoring studies was further established by the reaction between 3-(o-chlorobenzoyl) benzothiazoline-2-thione (4c) with aniline, o-aminophenol and ethanolamine (Fig. 5.7).
From the foregoing figures, as expected, the structure of amines has profound influence on the rate of the reaction. Aminophenols in which the electron density at the amino group is high reacts with 3-benzoyl benzothiazoline-2-thione and 3-(o-chlorobenzoyl) benzothiazoline-2-thione, faster than aniline and ethanolamine. However, the reaction with 3-(phenylacetyl) benzothiazoline-2-thione shows a different trend. In all the three examples ethanol amine shows least reactivity.
Attempts were also done to know whether there is any effect for the attached activated carboxyl group on the rate of the reaction. For this, 3-benzoyl benzothiazoline-2-thione (4a), 3-(phenylacetyl) benzothiazoline-2-thione (4b), and 3-(o-chlorobenzoyl) benzothiazoline-2-thione (4c), were reacted with aniline, o-aminophenol and ethanolamine. The reactions were monitored spectrophotometrically and graphs were plotted. (Fig. 5.8; 5.9 & 5.10). It is observed that, when a bulky group like o-chlorobenzoyl is attached on 2-mercaptobenzothiazole (1), the reaction is very slow.

Fig. 5.8 Reaction of 3-acyl benzothiazoline-2-thiones with aniline
Fig. 5.9 Reaction of 3-acyl benzothiazoline-2-thiones with ethanolamine

Fig. 5.10 Reaction of 3-acyl benzothiazoline-2-thiones with o-aminophenol
Apart from the above studies, the effect of solvent on the rate of aminolysis was also carried out. Here four different solvents were tried on the same reaction. Thus 3-benzoyl benzothiazoline-2-thione (4a) was treated with aniline in different solvents like chloroform, ethanol, methylene chloride and ethyl acetate. The aminolysis reaction was monitored and graphs were plotted using different solvents. It was observed that the reaction in methylene chloride was so sluggish and irregular that made plotting very difficult. However, plots were drawn for the reactions in ethanol, chloroform and ethyl acetate as solvent (Fig. 5.11). From the plot, it is evident that the reaction is very fast in chloroform and ethanol. Though, ethanol is much superior, the possibility of side reaction under photochemical conditions poses problems. Therefore, it can be concluded that the aminolysis reaction of 3-acyl derivatives of 2-mercaptobenzothiazole is favoured in chloroform solvent.

5.2.3 UV-visible spectrum of 3-acyl benzoxazoline-2-thione

The absorption band in the UV-visible spectrum of 2-mercaptobenzoxazole (2) in chloroform is found to be at 302 nm (Fig. 5.12). When these thiol is derivatized with acids
Fig. 5.11 Reaction of 3-benzoyl benzothiazoline-2-thione in different solvents

and UV-visible spectrum taken at the same dilution shows that the band at 302 nm is slightly shifted to a new wavelength 293 nm together with considerable decrease in the intensity of absorption (Fig. 5.13).

5.2.4 UV-visible spectrum of 3-acyl benzoxazoline-2-thione during aminolysis

When acyl derivatives of 2-mercaptobenzoxazole are treated with amines, 2-mercaptobenzoxazole (2) is regenerated along with the amides. UV spectrum shows that
Fig. 5.12  UV-visible spectrum of 2-mercaptobenzoazole

Fig. 5.13  UV-visible spectrum of 3-benzoyl benzoxazoline-2-thione
during the reaction the characteristic band at 302 nm is regenerated and at the same time the intensity increases. Therefore spectrophotometric monitoring of aminolysis reaction is possible similar to the aminolysis of 3-acyl benzothiazoline-2-thione.

Thus, a very dilute solution of 3-benzoyl benzoxazoline-2-thione in chloroform was mixed with an equivalent amount of aniline in chloroform and stirred well. A definite quantity of the mixture was withdrawn at regular intervals of time (1 min) and absorbance was measured spectrophotometrically. As the reaction proceeds, the intensity of the peak at 302 nm increases gradually and reaches a limiting value (Fig. 5.14). The band at 302 nm is due to the regeneration of 2-mercaptobenzoxazole during the reaction, which helps to measure the extent of reaction.

![Fig. 5.14 UV-visible spectrum of 3-acyl benzoxazoline-2-thione during aminolysis](image-url)
A quantitative correlation was made by measuring the absorbance at the wavelength of maximum absorption. For this the mixture was regularly introduced into the spectrophotometer at intervals and the absorbance was measured till constant values were obtained. Absorbance at infinite time (1 h) was also taken. A graph was plotted by taking relative absorbance ($D_{rel}$) on Y-axis and time on X-axis.

A plot of the reaction between 3-benzoyl benzoxazoline-2-thione (5a) and aniline is given in Fig. 5.15. The reaction was very fast and abruptly reaches 80% conversion within two minutes and after that a gradual one. The above spectrophotometric monitoring was extended to other amines such as ethanol amine and diphenyl amine (Fig. 5.15). In both the cases within four minutes more than 90% conversion has taken place.

Analogous monitoring studies were also carried out with 3-(o-chlorobenzoyl) benzoxazoline-2-thione (5b) and different amines (Fig. 5.16). Similar to 3-benzoyl benzoxazoline-2-thione, the reaction with aniline is very fast. Within two minutes the reaction reaches 80% completion and after that attains the limiting value.
Fig. 5.15 Reaction of 3-benzoyl benzoxazoline-2-thione with amines

However, the reaction with ethanolamine is sluggish and with diphenylamine is very very slow. In the case of diphenylamine, it required seventeen minutes for 90% conversion.

The reaction of 3-acyl derivatives of 2-mercaptobenzoxazoles with o-aminophenol was very interesting. In this, 3-benzoyl benzoxazoline-2-thione and 3-(o-chlorobenzoyl) benzoxazoline-2-thione react with o-aminophenol very quickly and reaches 97% conversion within two minutes (Fig. 5.17).
Fig. 5.16 Reaction of 3-(o-chlorobenzoyl)benzoxazoline-2-thione with amines

Fig. 5.17 Reaction of 3-acyl benzoxazoline-2-thiones with o-aminophenol [a - benzoyl; b - o-chlorobenzoyl]
From the plots, as expected, the structure of amines plays an important role on the rate of the reaction. 2-Aminophenol which is highly nucleophilic reacts faster than the other three amines. Out of the other three amines, aniline which is more nucleophilic reacts faster than the other two. The reaction with ethanolamine is slow compared to aniline and the very much sluggish nature of diphenylamine may be attributed to the bulkness of the group.

Just similar to 3-acyl benzothiazoline-2-thione, attempts were also done to know whether there is any effect for the attached activated carboxyl group on the rate of the reaction. For this 3-benzoyl benzoxazoline-2-thione (5a) and 3-α-chlorobenzoyl benzoxazoline-2-thione (5e) were treated with ethanolamine and diphenylamine. The reactions were monitored spectrophotometrically and graphs were plotted (Fig. 5.18 & 5.19). Analogous to 3-acyl benzothiazoline-2-thione (4) when a bulky group like o-chlorobenzoyl is attached to 2-mercaptobenzoxazole (1), the reaction is found to be very low.

The effect of solvent on the rate of aminolysis reaction was also investigated. For this seven different solvents were tried on the same reaction. Thus 3-benzoyl
Fig. 5.18  Reaction of 3-acyl benzoxazoline-2-thiones with ethanolamine

Fig. 5.19  Reaction of 3-acyl benzoxazoline-2-thiones with diphenylamine
benzoxazoline-2-thione (5a) was treated with aniline in ethanol, chloroform, benzene, ethyl acetate, CCl₄, dioxan and methylene chloride. The aminolysis reactions were monitored and graphs were plotted. Table 5.1 gives the $\lambda_{\text{max}}$ values of the aminolysis reactions using different solvents.

Table 5.1. $\lambda_{\text{max}}$ of the aminolysis reactions in different solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Benzene</td>
<td>357</td>
</tr>
<tr>
<td>2. Carbon Tetra Chloride</td>
<td>325</td>
</tr>
<tr>
<td>3. Chloroform</td>
<td>300</td>
</tr>
<tr>
<td>4. Dioxan</td>
<td>330</td>
</tr>
<tr>
<td>5. Ethanol</td>
<td>320</td>
</tr>
<tr>
<td>6. Ethyl acetate</td>
<td>328</td>
</tr>
<tr>
<td>7. Methylene chloride</td>
<td>330</td>
</tr>
</tbody>
</table>

The $\lambda_{\text{max}}$ maximum values of thiol regenerated using different solvents were plotted against time (Fig. 5.20). From the graph it is evident that aminolysis reaction in ethanol and chloroform medium are very fast. In ethyl
acetate and CCl₄, the reactions are slow and require nearly 15 min for the 75% conversion. In dioxan and methylene chloride, the reaction is still slow and it requires about 30 min for 75% conversion. Out of the different solvents used, methylene chloride gave the least reaction rate. Hence it is evident that both in 3-acyl benzothiazoline-2-thiones and 3-acyl benzoxazoline-2-thiones, the aminolysis reaction is favoured in the solvent chloroform. (Ethanol is a nucleophile and therefore cannot be suggested as a solvent).

Fig. 5.20 Reaction of 3-benzoyl benzoxazoline-2-thione with aniline in different solvents
Finally, attempts were done in order to compare the reactivities of the 3-acyl benzo(thiazoline/oxazoline)-2-thione (4 & 5) on the basis of spectrophotometric measurements. Equimolar solutions of 3-benzoyl benzothiazoline-2-thione (4a) and 3-benzoyl benzoxazoline-2-thione (5a) were dissolved in chloroform and separately treated with aniline and ethanolamine (Fig. 5.21 and 5.22). It is clear that aminolysis reaction with 3-acyl benzothiazoline-2-thione are slow compared to 3-acyl benzoxazoline-2-thione.

Fig. 5.21 Reaction of aniline with (a) 3-benzoyl benzoxazoline-2-thione and (b) 3-benzoyl benzothiazoline-2-thione
Fig. 5.22 Reaction of ethanol amine with (a) 3-benzoyl benzoxazoline-2-thione and (b) 3-benzoyl benzothiazoline-2-thione

5.3 Experimental

For the spectrophotometric monitoring, the reactions were conducted in the cuvet of the UV-visible spectrophotometer. Here a dilute solution (1 mmol) of 3-acyl benzo(thiazoline/oxazoline)-2-thione was prepared in solvents like chloroform, ethanol, ethylacetate, carbon tetrachloride, dioxan, benzene and methylene chloride separately. 1 Ml of this solution was diluted to 100 ml using a standard flask. Similarly 0.01 mmol dilute solution of different amines
(aniline, benzylamine, ethanolamine, o-aminophenol etc.) were prepared. 1 Ml of the above dilute solution of 3-acyl benzo(thiazoline/oxazoline)-2-thione was taken in the cuvet and the absorbance was measured initially. Dilute solution (1 ml) of the amine was then transferred to the cuvet, stirred well with the aid of a capillary tube and the absorbance was noted immediately. This absorbance value was treated as D0 (initial time). The reaction mixture was taken outside, stirred well and absorbance measurements were done at regular intervals (1 minute). Here the time taken for experimental operation and stirring was neglected.
PEPTIDE SYNTHESIS