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Studies on Kinetics of Colour Formation of Asparagine - Ninhydrin Complex

Ashish Kumar Sharma[1], G. D. Nigam[2], Sudhanshu Mohan[3]

Chemistry Department, DAV PG College, Civil Lines, Kanpur-208001
drsmsinha[at]gmail.com

Abstract: ***The kinetic studies of Ninhydrin (120 ppm) and Asparagine (100-1000-ppm) were undertaken at different temperatures viz. 75°C, 85°C and 95°C. The reaction of complexation was found to be of first order. The average rate of reaction for 120 ppm of Ninhydrin and 100 ppm of Asparagine was found to be 0.8529 x 10^{-2} min^{-1} 0.9394 x 10^{-2} min^{-1} and 1.0830 x 10^{-2} min^{-1} at 75°C, 85°C and 95°C respectively. Similarly for Ninhydrin 120 ppm and Asparagine concentration of 1000 ppm the rate constant of complexation at 75°C, 85°C and 95°C was observed to be 1.7919 x 10^{-2} min^{-1}, 2.7047 x 10^{-2} min^{-1} and 2.7818 x 10^{-2} min^{-1}, respectively.***

Keyword: Kinetics, Ninhydrin-Asparagine, Complexation, Reaction Order, First order Reaction.

1. Introduction

The reaction of ninhydrin with primary amino groups to form the purple dye now called Ruhemann's purple (RP) was discovered by Siegfried Ruhemann in 1910. In addition, imines such as, pipecolic acid and proline. Since its discovery, extensive efforts have been made to apply manual and automated ninhydrin reactions as well as ninhydrin spray reagents to the detection, isolation, and analysis of numerous compounds of interest across a broad spectrum of disciplines. These include agricultural, biochemical, clinical, environmental, food, forensic, histochemical, microbiological, medical, nutritional, plant, and protein sciences. This reaction is unique among chromogenic reactions in that at pH 5.5 it results in the formation of the same soluble chromophore by all primary amines which react, be they amines, amino acids, peptides, proteins, and even ammonia. Because the chromophore is not chemically bound to the protein or other insoluble material, it is not lost when the insoluble substrate is removed by centrifugation or filtration after the reaction is completed. The visible color of the chromophore is distinctive and is generally not affected by the yellow colors present in many foods, plant, and tissue extracts. Kinetically the study of reaction was taken up by researchers, out of which some important works are given as under.

Mendel Friedman & Carl Ul Sigel[1] studied the reaction rate of α-Amino acids with Ninhydrin at 30 & 100°C to calculate the separate polar and steric parameter that influence the rate of reaction. Kabir-ud-Din et al.[2] studied reaction of Ninhydrin with DL-methionine in absence and presence of organic solvents (Propanol dimethyl sulfoxide, acetonitrile and methyl cellosolve) and observed that addition of each solvent increase the absorbance as well as rate constant. The reaction follows first order and fractional order kinetics with respect to [Ninhydrin] and [H^+] respectively in the excess of methionine. Dileep Gupta et al.[3] studied the kinetics and mechanism of Ninhydrin reaction with Cu (glycine) and Cu (alanine) in the ratio of 1:1. The kinetics were found to follow psuedo-first order reaction. G.D. Nigam4 studied the kinetics of Ninhydrin, Aspartic acid complex at different temperatures 25°, 55° & 75°C and varying concentration of Aspartic acid (100 ppm to 1000 ppm) and found reaction to be of first order.

The present studies aim at kinetic study of Ninhydrin-Asparagine complexation at 75°C, 85°C and 95°C at varying concentration of Asparagine 100 to 1000 ppm with fixed concentration of Ninhydrin (120 ppm).

2. Methodology

Ninhydrin solution – For the repeated use of ninhydrin, stock solution of 500 ppm was prepared by dissolving Ninhydrin (Thomas Baker) in double distilled water.

Asparagine solution – Standard Asparagine stock solution of 1000 ppm was prepared by dissolving required quantity of Asparagine A.R. Grade (Merck) in double distilled water in 1L volumetric flask.

The optimum quantity Ninhydrin was determined by taken 100 ppm of Asparagine solution and varying the quantity of Ninhydrin from 20 ppm to 200 ppm by adding required amount of Ninhydrin from stock solution in volumetric flask numbered 1-10. The solutions were mixed thoroughly and made up to 100 ml. with double distilled water. These Stoppard flasks were kept in water bath maintained at 95°C for 60 min., then the solution was allowed cool to room temperature and absorbance was measured at 568 nm by the help of Spectrophotometer (Chimeto-2375 Double beam spectrophotometer, India). The 120ppm of ninhydrin showed the maximum value of absorbance and further increase in concentration dose not made any noticeable increase in the value of colour.

This 120ppm quantity of Ninhydrin was fixed and increasing amount of Asparagine (100 ppm to 1000 ppm) were added to the volumetric flask numberd 1-10. The solutions were mixed thoroughly and made upto 100 ml by adding a double distilled water (Conductance 0.5 to 1.5 micro siemens) these sets were kept in water at 75°C for 60 min after cooling the mixture to room temperature the coloured was measured at 568 mm. Similar experiment as maintained above was performed at 85°C and 95°C. Detailed investigations were

therefore undertaken to study the behavior of Ninhydrin and Asparagine complex.

The kinetics of colour formation in pure solution of Asparagine and Ninhydrin complex was studied by dissolving required quantity of C.P. Grade Asparagine and Ninhydrin in double distilled pure water separately. The studies in pure solutions were made at 75°C, 85°C and 95°C.

The temperature at the reaction vessel was maintained with the help of the Hoppler Thermostat (NBE type German Make), which maintained temperature with in ± 0.1°C. The heaters in the bath were energized by electric relay circuit. The Ninhydrin and Asparagine solutions were prepared in double distilled water & mixed in desired quantity in a test tube fitted with caps. The progress of the reaction was followed by taking out reaction mixture at definite interval of times and measuring the colour on 2375 double beam spectrophotometer at 568 nm.

3. Results and Discussion

The results on the kinetics of colour formation of Ninhydrin (120 ppm) and Asparagine (100-1000 ppm) in a aqueous solution at 75°C, 85°C and 95°C. The order of reaction[5-7] was determined by two methods.

(i) Integration method (calculation method)
The first order reaction equation is as follows:

$$K = \frac{2.303}{t} \log_{10} \frac{a}{(a-x)}$$

K = Specific reaction rate constant,
a = initial concentration of Asparagine α the maximum absorbance value at 568 nm.
x = Concentration of Asparagine that has reacted with Ninhydrin
$(a-x)$ = Remaining concentration of Asparagine at time (t)

Table-1 show the K values calculated for various concentrations of Asparagine (100 - 1000 ppm) and Ninhydrin (120 ppm fixed) at three distinct temperatures, namely 75, 85 and 95°C. The values of K are reasonably constant at certain temperature and concentration, as seen in the table. This consistency was observed over the whole temperature range investigated.

Table 1: Average value of the reaction rate constant in min^{-1} x 10^{-2} for Asparagine- Ninhydrin complexation at various temperatures

Conc. of Asparagine (ppm)	Rate constant in min^{-1} x 10^{-2}, temp. °C		
	75°C	85°C	95°C
100	0.8529	0.9394	1.0830
200	0.9134	1.1394	1.2969
300	0.9433	1.2207	1.3674
400	0.9821	1.8594	2.1667
500	1.2108	2.0977	2.3019
600	1.4248	2.2103	2.3987
700	1.5899	2.4125	2.5069
800	1.7356	2.5809	2.6393
900	1.6704	2.6255	2.5436
1000	1.7919	2.7047	2.7818

The average values of K increased as the temperature and Asparagine concentration increased. Thus with a 100 ppm Asparagine concentration, the average 'K' value at 75°C, 85°C & 95°C were 0.8529 x 10^{-2}, 0.9394 x 10^{-2} and 1.0830 x 10^{-2} min^{-1}, respectively.

For all Asparagine concentration, a comparable increase in the value of 'K' was found (100-1000 ppm). At a constant temperature, the value of 'K' also increased with concentration, as indicated in Table-1.

(ii) Graphical method
The plot log (a-x) vs time (t) was used in the graphical method for determining order of reaction fig. 1,2 and 3 show a linear curve for concentration 100 ppm and 1000 ppm of Asparagine at 75, 85 and 95°C, respectively, indicating the applicability of the first order reaction. 'K' was calculated using the slope of the straight line.

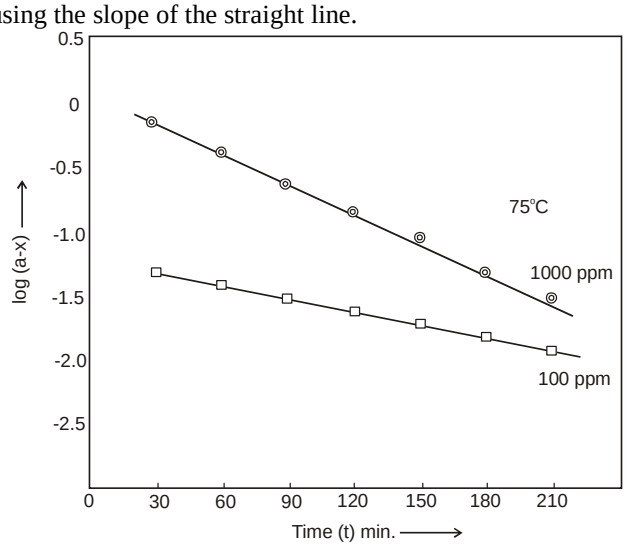

Figure 1: Variation of colour as function of time at 75°C

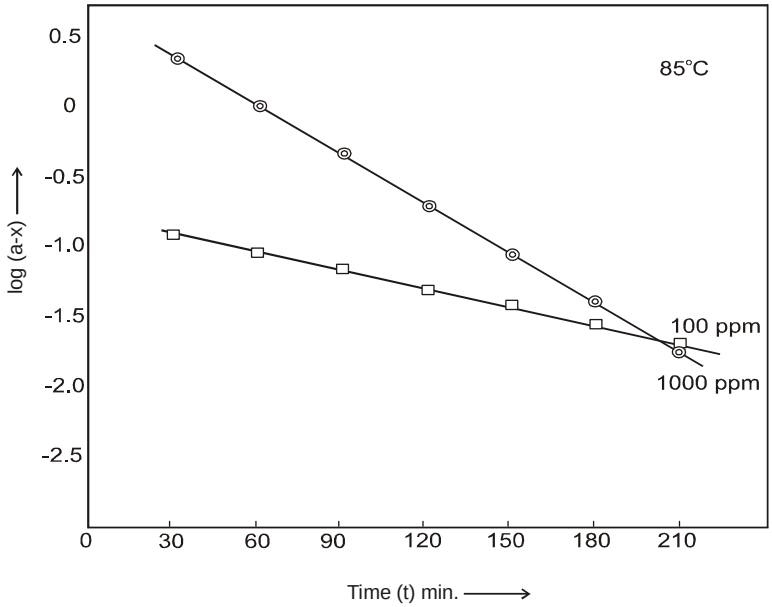

Figure 2: Variation of colour as function of time at 85°C

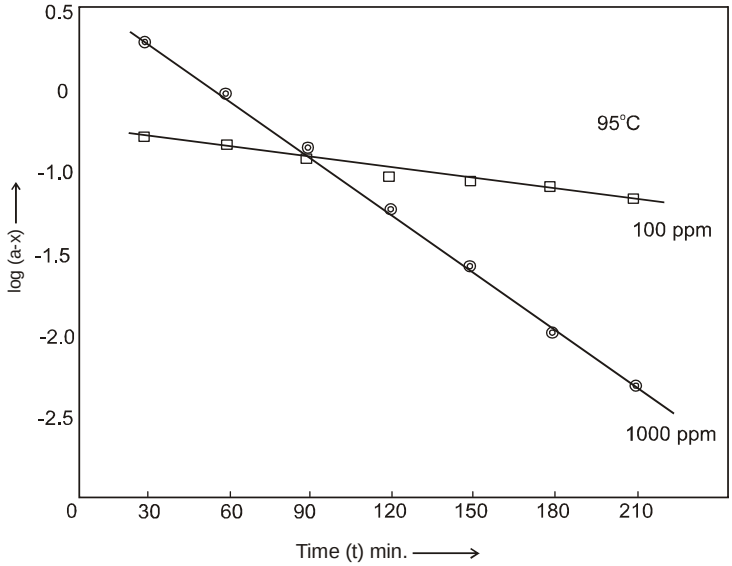

Figure 3: Variation of colour as function of time at 95°C

The plots were linear, the value of K computed from the slope of the plot as shown in Fig. 1, 2 and 3, were 0.822 x 10^{-2}, 1.007 x 10^{-2}, and 0.9691 x 10^{-2} min^{-1} for 100 ppm Asparagine at 75°C, 85°C and 95°C and 1.9191 x 10^{-2}, 2.7095 x 10^{-2} and 2.7417 x 10^{-2} min^{-1} for 1000 ppm of Asparagine at 75°C, 85°C and 95°C, respectively. It should be noted that above values are representative of a set of studies conducted to determine the rate constant of reaction.

Table 2: Comparative data of rate constant obtained by calculation and graphical method

Conc. of Asparagine (ppm)	Rate constant in min-1 (K x 10^2)		
	Temp. 75°C	Temp. 85°C	Temp. 95°C
Calculation method			
100	0.8529	0.9394	1.0830
1000	1.7919	2.7047	2.7818
Graphical method			
100	0.8220	1.00756	0.9691
1000	1.9191	2.7095	2.7417

Table 2 shows a comparison of reaction rate constant data obtained using the calculation method and graphical method. The table shows that the results obtained by calculation and graphical methods are nearly identical with in the margins of experimental error. Thus it may be concluded that reaction of Asparagine and Ninhydrin complex followed first order kinetics.

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