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Studies on Methods for Determination of Asparagine in Sugar House Products

Ashish Kumar Sharma, G. D. Nigam, S. Mohan*

drsmsinha[at]gmail.com
Chemistry Department, DAV PG College, Kanpur-208001, India
National Sugar Institute, Kanpur-208017, India

Abstract: ***The present work is aimed at finding out suitable method for determination of Amino-acids in cane juice, syrup, massecuite and molasses particularly Asparagine. The Asparagine-Ninhydrin complex was found to exhibit maxima at 568 nm. As also reported amino acids with ninhydrin yielded Ruhemann purple and the corresponding uv-visible spectra had absorption maxima around 405 and 570 nm. The effects of several factors including the reaction temperature, the ninhydrin concentration, and pH on the asparagine-ninhydrin reaction were investigated to optimize the specificity and sensitivity. As a result, a simple and specific colorimetric asparagine assay was developed. It was observed that Asparagine developed color with Ninhydrin at 75^{o}C and above in contrast to other amino acids which could develop color at lower temperatures viz.25^{0}C and 55^{o}C. The absorbance values increased from 0.0266 at 75^{o}C to 0.0685 at 85^{o}C and 0.3349 at 95^{o}C for 100 ppm. of Asparagine. It was also observed that absorbance values for fixed.100 ppm to 1000 ppm. increased up to 700 ppm or 800 ppm of Asparagine then attained almost a constant value.***

Keywords: Asparagine, Ninhydrin, Ruhemann's Purple

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 [1] 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. Adaptations of the classical ninhydrin reaction to specialized needs in analytical chemistry and biochemistry include the use of acid, alkaline, and fluorogenic ninhydrin reagents. To cross-fertilize information among several disciplines wherein an interest in the ninhydrin reaction has developed, and to enhance its utility, this review attempts to integrate and correlate the widely scattered literature on ninhydrin reactions of a variety of structurally different compounds. Specifically covered are the following aspects: historical perspective, chemistry and mechanisms, applications, and research needs. A better understanding of these multifaceted ninhydrin reactions provides a scientific basis for further improvements of this important analytical technique.

A large number of methods are involved and available in literature for the estimation of Amino acids. These were reviewed thoroughly and methods particularly employed for estimation of asparagine were critically studied in order to select a method, which may be free from the interference of other impurities in technical sugar solution. Some of these are thin layer chromatography method [2, 3], HPLC method [4], Ninhydrin calorimetric method [5-9] etc.

Review of literature and with the study of all above three methods it was inferred that Ninhydrin calorimetric method is most suitable for determination of Asparagine in the sugar house products. The temperature in the sugar house during the processing ranges from 65 to 102^{o}C so the method was tried to get the best result in this temperature range only.

2. Materials and Methods

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.

Method: 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^{o}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.

Determination of effect of increasing quantity of Asparagine after optimizing the quantity of Ninhydrin to 120ppm. 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.

3. Results and Discussion

Absorbance maxima of Asparagine-Ninhydrin complex. The spectra of complex formed shows a maxima at 568 nm. It was observed that absorbance increased with increase in wavelength. It was maximum at 568 nm. and then started decreasing as the wavelength was increased from 568 to 720 nm. and attained minimum value at 800 nm Figure 1.

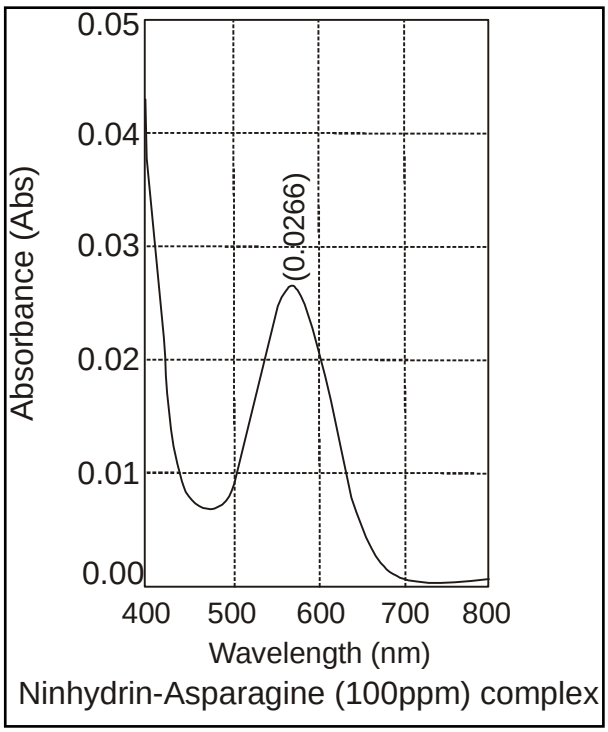

Figure 1: Visible range spectra of Asparagine-Ninhydrin complex in water

Complex of Amino Acid-Ninhydrin in cane juice-The plot of absorbance against wavelength showed maxima at 568 nm, which is characteristic maxima of pure solution of Asparagine in Ninhydrin as shown in Figure 2.

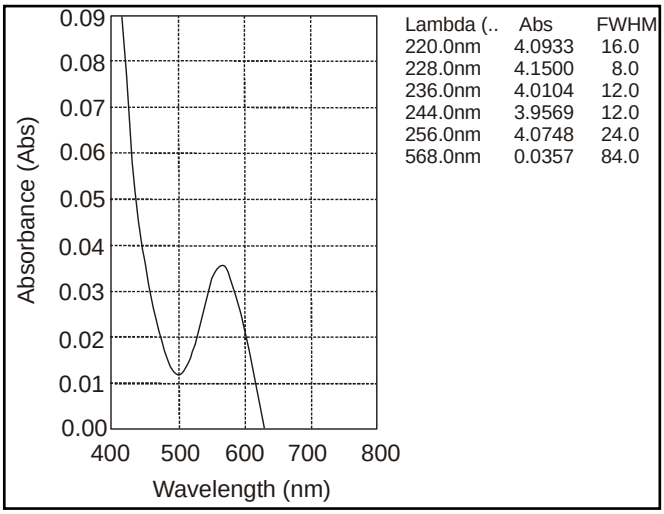

Figure 2: Visible range spectra of Asparagine-Ninhydrin complex in Cane juice

It is evident from the above observations that maximum remains unaffected in presence of other number of Amino acids present in cane juice. This also leads to inference that Asparagine is present in large amount in cane juice as compared to the other Amino-acids. The above findings also suggest that Ninhydrin method can be used for determination of Amino acids in cane juice and different sugar-house products obtained during processing of cane juice into crystal sugar.

Optimum concentration of Ninhydrin-Amino acids complex in cane juice:

The experiments were conducted by using increasing concentration of Ninhydrin with different concentration of Amino-acids. It was observed that with increase in concentration of Ninhydrin the absorbance values increased upto 120 ppm of Ninhydrin. Further, increase in concentration of Ninhydrin decreased values of absorbance were observed. These results showed that 120 ppm concentration of Ninhydrin is the optimum for complete complexation of Amino-acids of cane juice.

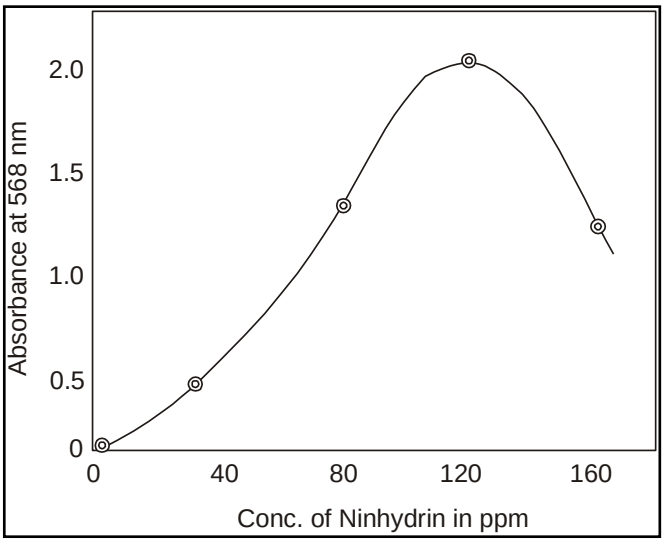

Figure 3: Optimum concentration of Ninhydrin for complete complexation of Amino-acids of cane juice

Effect of temperature on the Asparagine-Ninhydrin complexation:

It was observed during the initial experiments conducted at different temperature, that the temperature viz.25°C, 55°C and 75°C did not develop colour. The temperatures were raised to 75°C, 85°C and 95°C which in reality is also the working temperature in sugar house during the processing of sugar in industry. It was observed that temperature between 85°C to 95°C developed significant colour. The use of increased temperature beyond 95°C did not yield any appreciable increase in colour. It was therefore decided to study the colour development at 75°C, 85°C and 95°C with increasing concentration of Asparagine (100 ppm to 1000 ppm) with optimum concentration of Ninhydrin as 120 ppm.

Table-1 shows that the absorbance at 75°C increase from 0.02666 to 0.80 up to 800 ppm of Asparagine and thereafter becomes constant similarly at 85°C the absorbance was 0.0685 at 100 ppm and then increased to 3.8015 for 700 ppm and then become almost constant. The similar trends were observed at 95°C where the value increased 700 ppm of Asparagine and then become constant.

Table 1: Absorbance of Asparagine-Ninhydrin complex at 75°C, 85°C & 95°C

Conc. of Asparagine- (in ppm)	Temperature 75°C	Temperature 85°C	Temperature 95°C
100	0.0266	0.0685	0.3349
200	0.0340	0.6046	1.2527
300	0.1259	1.4522	2.5796
400	0.2145	2.6789	3.6280
500	0.4608	3.2535	3.9510
600	0.6250	3.6035	3.9920
700	0.7251	3.8015	4.0101
800	0.8010	3.9010	4.0205
900	0.8019	3.9020	4.0209
1000	0.8030	3.9110	4.0210

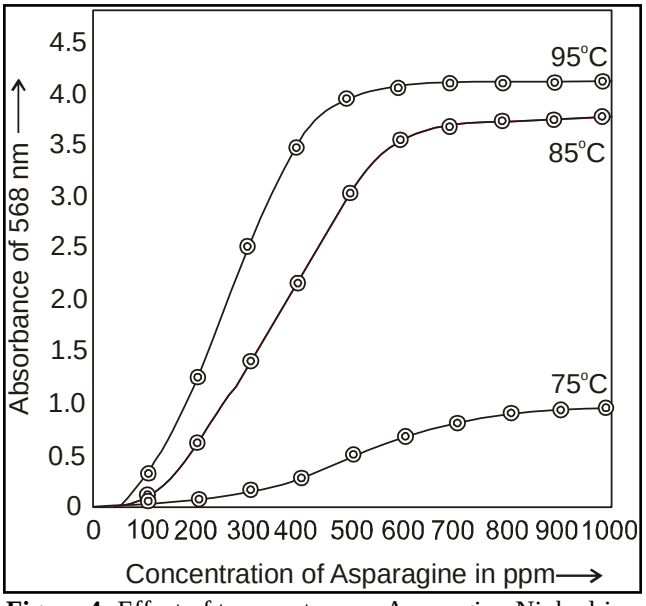

Figure 4: Effect of temperature on Asparagine-Ninhydrin complex

Effect of concentration of Asparagine on absorbance of complex at 75°C

Table 2: The effect of increasing concentration of Asparagine at 75°C

Asparagine (in ppm)	Absorbance
100	0.0266
200	0.0340
300	0.1259
400	0.2145
500	0.4608
600	0.6250
700	0.7251
800	0.8010
900	0.8019
1000	0.8030

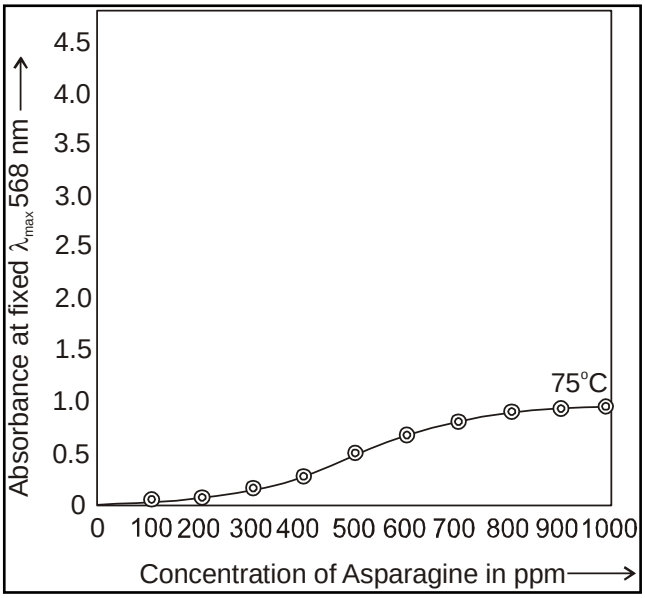

Figure 5: Effect of concentration of Asparagine on absorbance of complex at 75°C

Similar trends were observed at 85°C also where the absorbance values increased from 0.685 for 100 ppm of Asparagine to 3.9010 for 800 ppm.

Table 3: The effect of increasing concentration of Asparagine at 85°C

Asparagine (in ppm)	Absorbance
100	0.0685
200	0.6046
300	1.4522
400	2.6789
500	3.2535
600	3.6035
700	3.8015
800	3.9010
900	3.9020
1000	3.9110

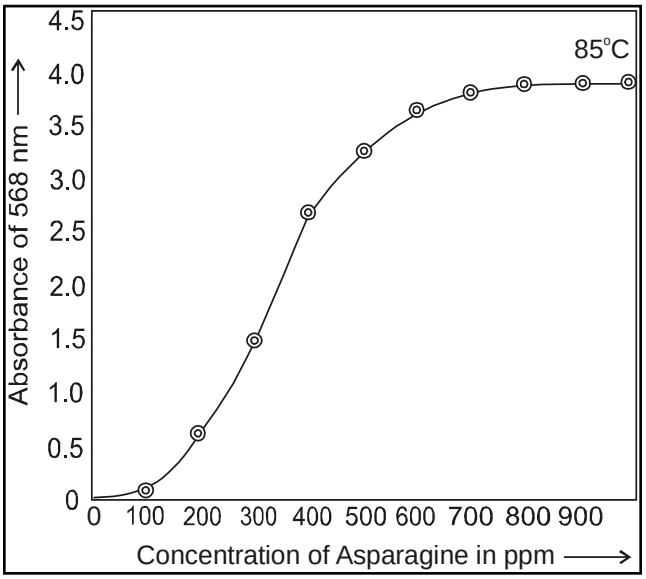

Figure 6: Effect of concentration of Asparagine on absorbance of complex at 85°C

It may be observed that at 95°C. The absorbance value varied from 0.3349 to 4.0101 for 100 ppm to 700 ppm of Asparagine, respectively.

Table 4: The effect of increasing concentration of Asparagine at 95°C

Asparagine (in ppm)	Absorbance
100	0.3349
200	1.2527
300	2.5796
400	3.6280
500	3.9510
600	3.9920
700	4.0101
800	4.0205
900	4.0209
1000	4.0210

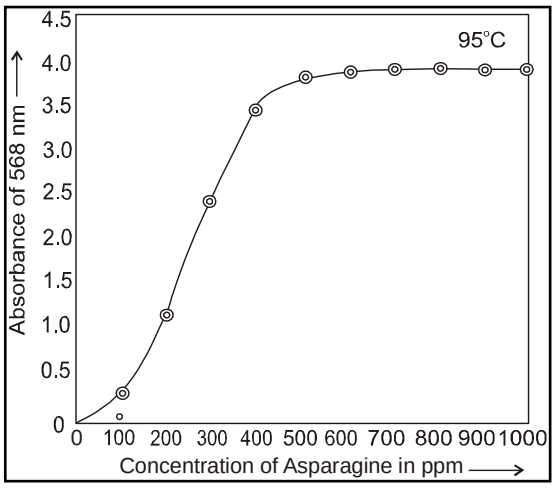

Figure 7: Effect of concentration of Asparagine on absorbance of complex at 95°C

Variation of absorbance as function of time during development of Asparagine-Ninhydrin complex

The absorbance of Asparagine-Ninhydrin complex at constant temperature 85°C increase linearly with increase in concentration of Asparagine (100-600 ppm) at different interval of time.

Asparagine (in ppm)	Absorbance (Asparagine-Ninhydrin) after		
	60 min	120 min	180 min
100	0.068	0.108	0.130
200	0.60	0.92	1.08
300	1.45	2.15	2.54
400	2.67	3.40	3.75
500	3.25	4.10	4.40
600	3.52	4.62	4.82

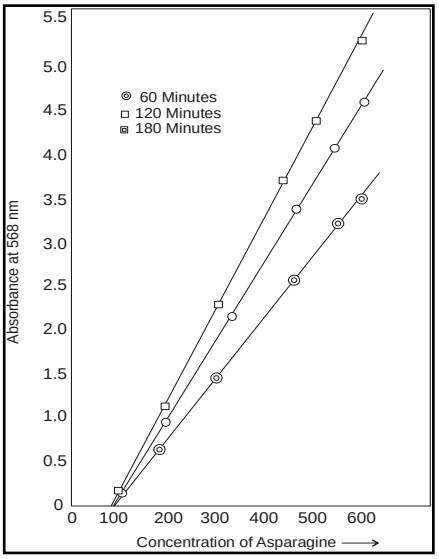

Figure 8: Absorbance vs concentration of Asparagine at different time intervals at 85°C.

4. Conclusion

This research work proved the effectiveness of the method applicable for the assessment of amino-acid in sugar house products. The above observation confirmed that Ninhydrin calorimetric method can be used for determination of Amino acids in sugar house products like juice, syrup etc. Application of this method to the sugar house products gives the quick and accurate assessment of amino-acid in sugar house products.

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