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Spectrophotometric determination of nicotinamide by the direct method using the reagent (4,3-diaminobenzaldehyde)

Sura Ismail Ibrahim[1)] , Qabas Naji Rashid[2)]
Department of Chemistry, College of Education for Girls , Tikrit University, Tikrit, Iraq. [a)]
Department of Pharmaceutical Chemistry, College of Pharmacy, Tikrit University, Tikrit, Iraq. [b)]
[a)]sura.ibrahim23@st.tu.edu.iq [b)]qabas.naji@tu.edu.iq

Abstract:

A new, simple, sensitive, and cost-effective method for determining nicotinamide in both its pure and pharmaceutical forms has been developed using spectrophotometric analysis with UV-visible spectrophotometry .The method involved an oxidative coupling reaction of both the drug and the organic reagent (4,3-Diaminobenzaldehyde) in an acidic medium using an oxidizing agent, and it yielded the highest absorption value at 398 nanometers. The linearity of the calibration curve was in the range of (10-160) micrograms/mL, while the molar absorptivity coefficient was (11074.656 L/mol/cm), with a detection limit of (1.53) micrograms/mL, and a quantification limit of (4.64) micrograms/mL. The method has been successfully applied for the determination of nicotinamide in both its pure form and pharmaceutical preparation .

Key words: Nicotinamide, oxidative coupling reaction ,$FeCl_3$,Spectrophotometric.

تقدير النيكوتيناميد طيفياً بالطريقة المباشرة باستخدام الكاشف (4,3 - داي امينوبنزالدهايد)

سرى اسماعيل ابراهيم [1)] أ.د. قبس ناجي رشيد [2)]
قسم الكيمياء، كلية التربية للبنات، جامعة تكريت، تكريت، العراق [أ)]
قسم الكيمياء الصيدلانية، كلية الصيدلة، جامعة تكريت، تكريت، العراق [ب)]

مستخلص:

تم تطوير طريقة جديدة وبسيطة وحساسة وفعالة من حيث الكلفة الاقتصادية لتقدير النيكوتيناميد في كل من صورتيه النقية وفي مستحضره الصيدلاني باستخدام التحليل الطيفي بمطيافة الأشعة فوق البنفسجية والمرئية. تضمنت طريقة التقدير بتفاعل الاقتران التأكسدي حيث تفاعل كل من الدواء والكاشف العضوي (4,3- داي امينو بنزالديهايد) في وسط حامضي باستخدام عامل مؤكسد، وكانت أعلى قيمة امتصاص للناتج المتكون عند 398 نانومتر. بينما كان نطاق خطية التركيز لمنحنى المعايرة في حدود (160-10) ميكروغرام/ مل، اما بالنسبة لمعامل الامتصاص المولاري (11074.656 ل/ مول/ سم)، مع حد كشف قدره (1.53) ميكروغرام/ مل، وكان حد الكمية قدره (4.64) ميكروغرام/ مل. وطبقت الطريقة التقدير هذه بنجاح لتقدير النيكوتيناميد في كل من شكليه النقي والمستحضر الصيدلاني طيفياً.

الكلمات المفتاحية : نيكوتيناميد ، تفاعل الاقتران التأكسدي ، كلوريد الحديد الثلاثي ، التحليل الطيفي.

INTRODUCTION:

Nicotinamide is used to treat niacin deficiency as it is considered one of the best treatments for this deficiency (1). Additionally, it is used to treat acne (2,3) because it has anti-inflammatory effects, as it increases the biosynthesis of ceramides and improves the permeability of the skin barrier in living organisms (4). It is effective in reducing the secretion of skin oils (5), and daily use reduces the risk of skin cancer (6,7). The frequent use of nicotinamide in general (8) has side effects including mild nausea, headaches, and slight digestive disturbances (9), and it is available in several forms (10,11) The dosage of nicotinamide is determined by several methods, such as UV.Vis-Spectrophotometer (12), HPLC (13), and GC(14), GL (15) .

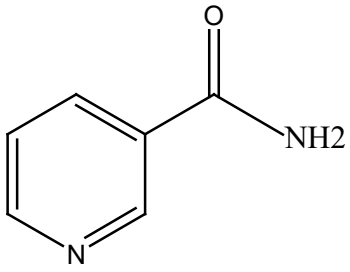

Fig. 1: Nicotinamide

The goal of the research was to find a method for estimating nicotinamide through an oxidative coupling reaction by oxidizing it using ferric chloride in an acidic medium. The method proved to be fast and simple, and it was used to estimate nicotinamide in both its pure form and pharmaceutical preparation.

EXPERIMENTAL:

Apparatus:

- T90 UV-Vis. Spectrophotometer.
- glass cell's1cm.
- Genesis UV10 Single-Beam UV-VIS Spectrometer.

The materials used :

- Nicotinamide 99% / SDI; Samarra. Iraq.
- H2SO4 99% / Fluka.
- HNO3 99,9% / Merck.
- HCl 37% / Scharlan.
- FeCl3/ Fluka.
- Diaminobenzaldehyde/ Merck

Solutions:

- The solution of nicotinamide: was prepared at a concentration of 1000 µg/mL by dissolving 0.1 grams in 100 mL of distilled water, and the other solutions were prepared by diluting the standard solution to the required con-

centration.

- Ferric Chloride :A weight of 0.1622 grams of ferric chloride was dissolved in 100 ml of water..

- HCl solution: At approximately 1.0 mol, 8.6 ml of concentrated acid (11.64 mol) is taken and diluted to a volume of 100 ml of distilled water.

- Diaminobenzaldehyde: A reagent solution was prepared by dissolving 0.134 grams of it in 100 ml of ethanol.

Process steps:

The optimal conditions were reached after conducting several preliminary tests for the oxidative coupling reaction of both the drug and the reagent in an acidic medium using an oxidizing agent. Specifically, 2 mL of the drug at a concentration of 500 µg/mL is transferred, followed by the oxidation of the drug by adding 1.0 mL of ferric chloride at a concentration of 0.01 M and adding 0.5 mL of hydrochloric acid. Then, a waiting period of 10 minutes is observed to complete the oxidation of the drug at room temperature. After that, 2.0 mL of the reagent (-4,3-diaminobenzaldehyde) at a concentration of 0.01 M is added, and the volume is then completed to the mark with distilled water, where the maximum absorbance of the colored product at 398 nanometers was 0.813. Figures (2) and (3).

Fig. 2: Absorption spectrum of Nicotinamide product against blank

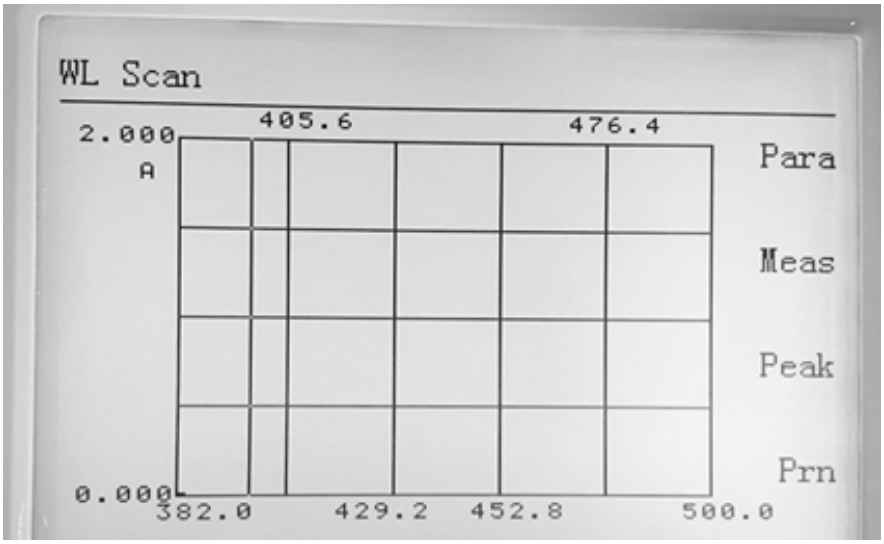

Fig. 3: Absorption spectrum of blank against distilled water

Optimal Conditions :

Study of acid volume and its effect on the product :

Effect of Reagent VolumeIncreasing volumes (0.5-4) mL of the reagent at a concentration of 0.01 molar were added to determine the effect of reagent volume on the absorption values of the product formed from the reaction, where the best absorption was at a volume of 2.0 mL., as shown in the figure (4).

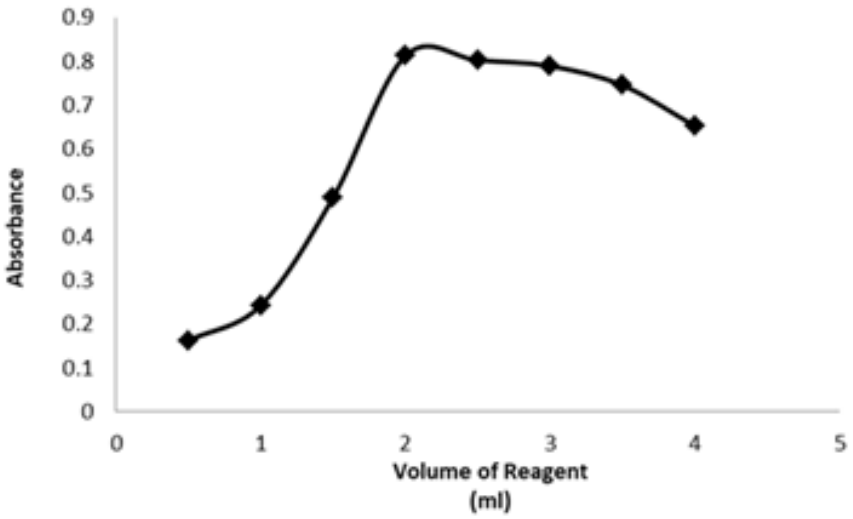

Fig 4. Effect of the optimal volume of the NAM-DAB product.

Study the effect of using different types of acids:

To determine which acid provides the best absorption when forming the product, different acids (H2SO4, HNO3, CH3COOH, HCl) were used, all at a concentration of 1.0 molar. It was found that hydrochloric acid gives the best absorption value, as shown in Table (1).

Table (1): Use of different acids and their effect on the absorption of the product

Acid	Abs.
HCl	0.814
HNO_3	0.108
H_2SO4	0.249
CH_3COOH	0.089

Study the effect of the oxidizing agent on the resulting product:

I used several increasing volumes of 0.3-2 mL of ferric chloride, and it was shown from the absorption values that the added volume of 1.0 mL is the best volume., as shown in Figure (5).

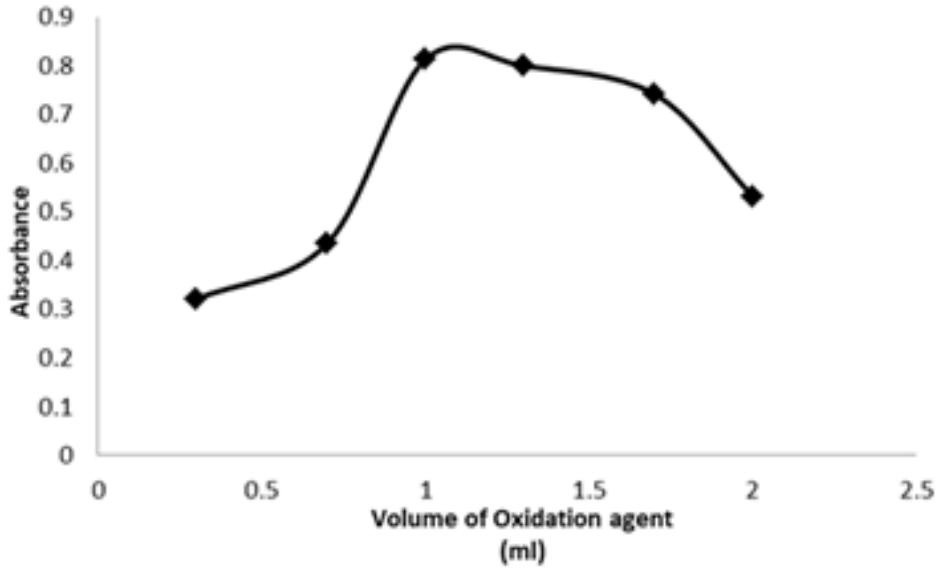

Fig. (5): Effect the volume of oxidation agent

Effect of Hydrochloric Acid Volume:

Increasing volumes (0.1-1.3) ml of acid at a concentration of 1.0 M were added to determine their effect on the absorption values of the product, and it was found that the best absorption of the formed product occurred at a volume of 0.5 mL. as shown in Figure 6.

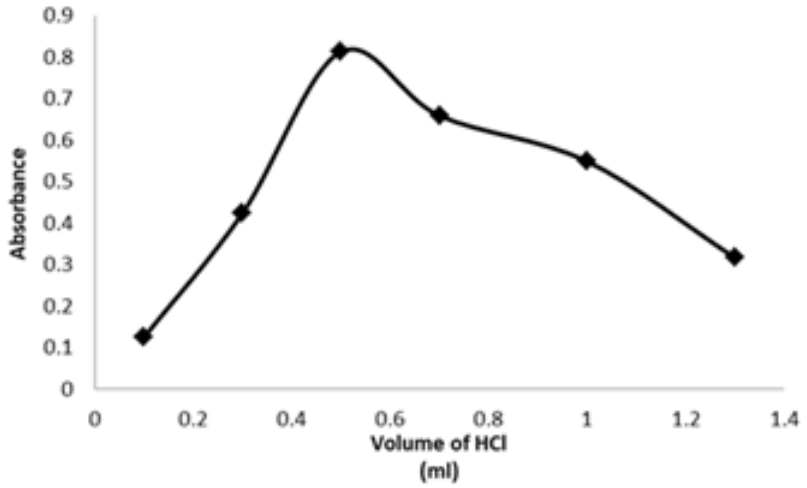

Fig. (6): Effect the volume of acid

Effect of Addition Sequence:

The effect of the addition sequence of the used solutions and its impact on the intensity of the resulting color was studied. Therefore, different experiments were conducted with different addition sequences, following the same conditions and optimal quantities in the subsequent experiments. **Table (2) shows** that sequence number (1) provides the highest absorption, so it was adopted in the subsequent experiments.

N	Addition sequence	Abs
1	R + A + O + D	0.811
2	R + O + A + D	0.359
3	D + A + R + O	0.317
4	D + R + A + O	0.166
5	R + D + O + A	0.105

(D) Nicotinamide and (R) 4,3-Diaminobenzaldehyde reagent and (O) Oxidizing agent Ferric Chloride and (A) Hydrochloric Acid

Study of Oxidation Time:

The effect of time on the completion of the oxidation process was studied by taking a series of samples, each containing 2.0 mL of the drug, to which 1.0 mL of the oxidizing agent and 0.5 mL of hydrochloric acid were added. The solutions were then left for different time periods before adding the reagent. After that, 2.0 mL of the reagent was added, and the absorption of the solutions was measured at a wavelength of 398 nanometers against their blank solutions. The results are **shown in Table (3)**, where it was found that a time of 10 minutes is sufficient to complete the oxidation process.

Time /minute	0	5	10	15	20	25
Abs.	0.805	0.809	0.812	0.812	0.813	0.812

Stability study of the formed output:

Knowing the stability of the produced output is very important, as it determines the duration for which the output remains constant. It has been shown that the absorption values of the produced output remain stable for up to 120 minutes The results are **shown in Table (4).**

Stability of the resulting output

Time /minute	0	15	30	45	60	90	100	120	150
Abs.	0.810	0.812	0.813	0.813	0.814	0.813	0.812	0.810	0.808

The effect of excipients in the pharmaceutical preparation:

The results obtained from the study of the effect of added substances (excipients) with Nicotinamide indicated that the excipients do not have any effect on the absorption value when producing the outcome, as two concentrations (200, 100) for each of the excipients were used, as **shown in Table (5).**

RE%	Added concentration (µg/ml)	RE%	Added concentration (µg/ml)	Additives
-1.11	200	-3.93	100	starch
3.32	200	-2.04	100	Magnesium carbonate
2.77	200	-1.86	100	Cellulose

Calibration Curve:

In the oxidation of the pure form of the drug with the organic reagent in an acidic medium, a linear calibration curve appeared in a concentration range of (10-160) micrograms/mL, **as shown in figure (7).**

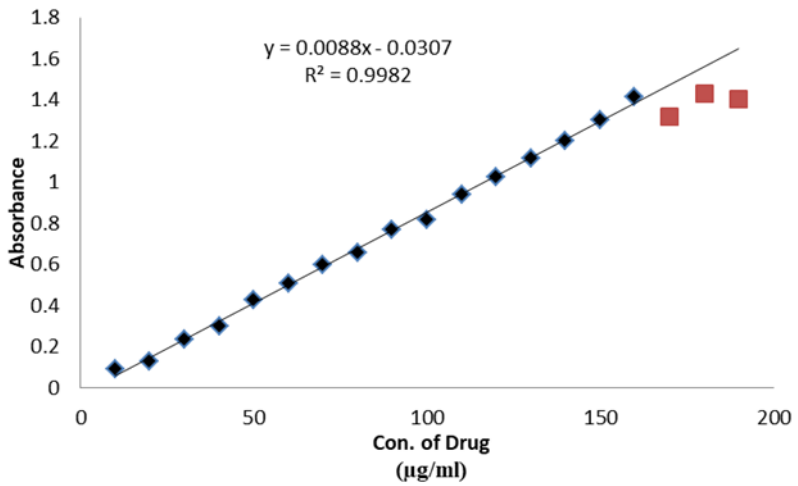

Fig. (7): Standard curve of the resulting product

Possibility of applying the studied method:

Determination of Nicotinamide in its pharmaceutical preparations (in tablet form). **Table (6).**

Table (6): Determination of NAM

Pharmaceutical preparation	Content (µg) declared	Found (µg) by proposed method	Recovery%
Safaplex	20	19.60	98.00
	60	59.98	99.97
	80	79.96	99.95

Study of accuracy and compatibility:

Calculating the accuracy and compatibility of the method by measuring three different concentrations of nicotinamide**, as shown in Table (7).**

RSD٪	RE٪	Concentration of the extract (µg/ml)	Concentration taken (mcg/ml)
2.72	-1.40	39.44	40
0.22	-1.57	59.06	60
0.12	0.81	90.73	90

Product equivalence study:

Determination of Stoichiometry The molar ratio method and the continuous changes method (Job's method) were applied, and both methods had a concentration of (10-3) molarity for both the drug and the reagent as shown in figures (8,9) .

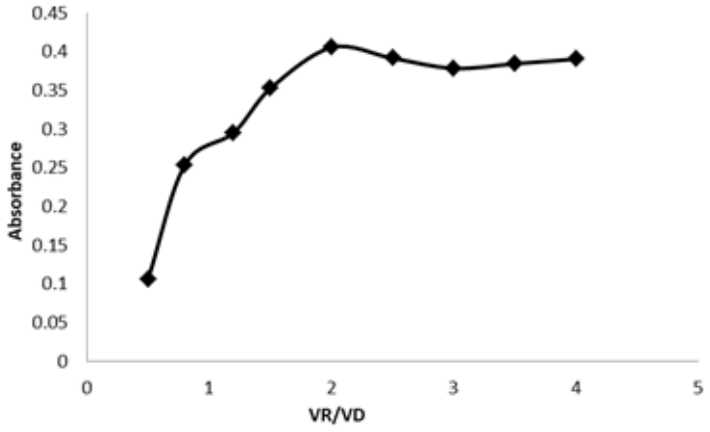

Fig. (8): Mole-ratio method of NAM-DAB

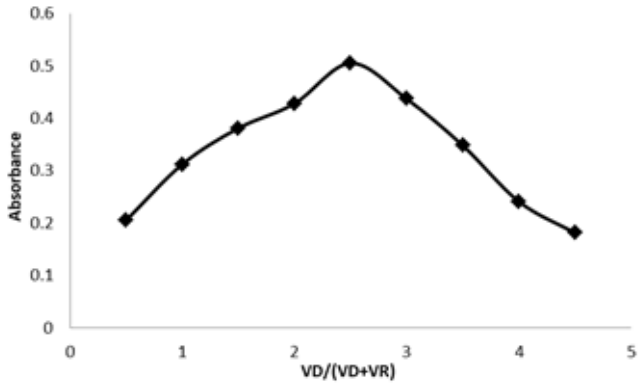

Fig. (9): Continuous variation method of NAM-DAB

Proposed reaction equation:

Proposed interaction according to the oxidation interaction and coupling between the drug and the organic reagent in the acidic medium (16) **as shown in Figure (10) .**

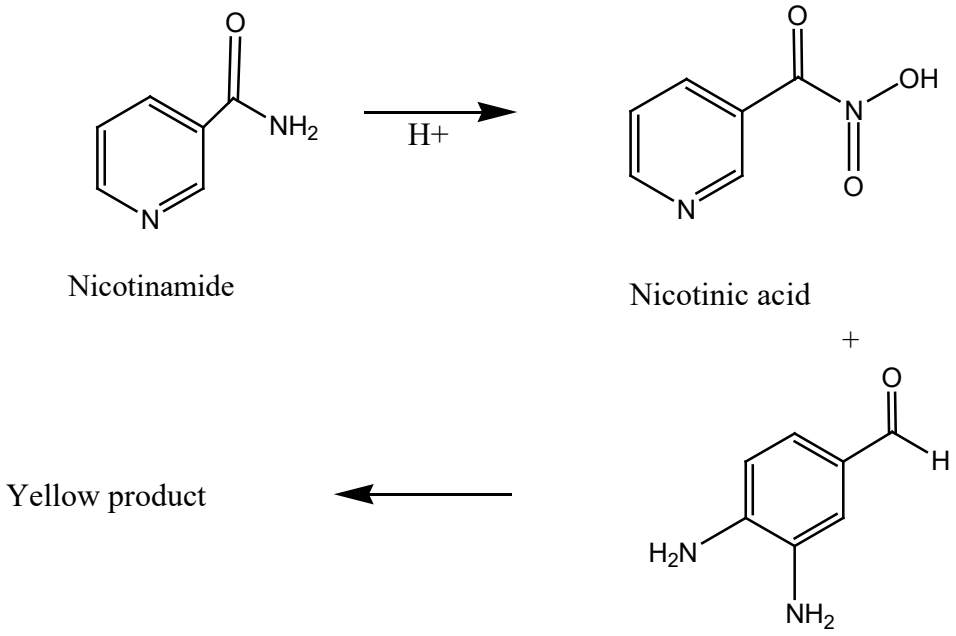

Fig. (10): Reaction equation

Table (8): Optical characteristics of the calibration curve of the formed product

Parameters	Value (crystal violat reagent)
λmax(nm)	398
Molar absorptivity (L/mol.cm)	1074.656
Correlation coefficient (r)	0.9991
Limit of Detection (µg/ml)	1.53
Limit of quantification (µg/ml)	4.64
Slope	0.0088
%RSD	0.28

CONCLUSION:

The proposed method is effective, quick, and cost-effective, and can be adopted for spectroscopic estimation of nicotinamide in both its pure form and pharmaceutical form, as it was conducted under simple and uncomplicated conditions. It showed good results.

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