Spectrophotometric assay of Nicotinamide in Pharmaceutical Dosages

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Abstract: The reaction of nicotinamide and alizarin reagent using charge transfer reaction at a pH of 5.54 lead to produce a red colored compound measured at 527 nm, while the blue colored complex was formed using the oxidation reduction reaction between nicotinamide and chromate at pH 3.49 in the presence of an indigo cochineal dye. These two colored products were measured at 527 and 610 nm respectively using two simple, fast and an accurate spectrophotometric methods. The linearity of the charge transfer method was followed Beer’s law 0.4 - 32 µg, while the oxidation reduction method was obeyed Beer’s law from 1.6 - 40 µg in depending on the concentration range. Molar absorptivity was 1.95×10^4 and 2.16×10^4 mol^{-1} cm^{-1} for the red and blue colored complex respectively. Finally, the values of Sandal’s sensitivity were 0.00626 and 0.00565 µg^{-2} cm^{-1} for the first and second methods respectively. These two methods have been applied to quantify the amount of nicotinamide in pharmaceuticals with good recovery.

Keywords: Nicotinamide, Assay, Alizarin, reaction, Indigo carmine, Dosage form.

1. Introduction
Nicotinamide (NAM), chemically named as 3-pyridinecarboxamide, vitamin PP, and vitamin B3.(Figure 1). There are two important sources of NAM, the first one is medication, and the second one is food supplements, like meat, vegetables, mushrooms, nuts, and found in trace amounts in fish. The niacin deficiency caused pellagra disease, which is treated with NAM, as well as NAM used for treated acne. The most important compounds NADH and NAD + were contain the important part of NAM [1-6].

Figure 1. Chemical structure of NAM

There are several methods have been used to estimate NAM in literatures, some of them are spectrophotometric methods for determination of NAM [7], also, chromatography methods were used for estimation NAM such as High-Performance Liquid Chromatography methods (HPLC)[8-9], HPTLC method[10], pre-column derivatization and high-performance liquid chromatography method combined with detection of fluorescence [11], High-Performance Liquid Chromatography method combined with UV detection [12].
In this paper, two organic reagents were used (Figure 2), the first is alizarin which was chemically named as 1,2-dihydroxyanthraquinone. Alizarin was derived from madder genus’ plant roots. The most important uses of alizarin as a prominent dye [13]. The second an organic reagent used in this project is indigo carmin dye, this dye is an oxidation reduction indicator. Indigo carmine chemically named with 5,5'-indigodisulfonic acid. The important uses of indigo carmine were a food colorant in industry, and in the manufacturing of capsule[15].

![Indigo Carmin dye and Alizarin](image)

**Figure 2.** Chemical structure of Indigo carmine and Alizarin

The aim of this project was to suggest spectrophotometric methods for the determination of NAM in pharmaceutical dosages, depending on charge transfer and oxidation reduction reactions.

### 2. Experimental:

#### 2.1. Equipment and chemical materials:

All the measurements of absorbance and final spectrum were carried out with JASCO–630UV-Visible spectrophotometer, and 1 cm matched cells. While the measurements of pH were carried out with HANA pH meter.

#### 2.2. Chemical materials:

The chemical solutions were prepared using chemical materials of an analytical reagent grade.

Nicotinamide solution, 1000 μg.mL⁻¹: To prepare a stock solution of NAM, 0.1000 g of NAM was dissolved in distilled water in a final volume of 100 ml using an dry and clean 100 mL volumetric flask. Then prepared from this solution NAM working solution of 100 μg/mL by appropriate dilution of stock solution with distilled water.

Alizarin solution 2.5×10⁻³ M: This solution was prepared when 0.0600 g of Alizarin (BDH) was dissolved with distilled water using a 100 mL volumetric flask.

Chromate solution, 8.6×10⁻⁴ M solution: this solution was prepared by dissolving 0.0167 g of potassium chromate (Fluka) in distilled water using a 100 mL volumetric flask.

Indigo Carmine, 1×10⁻³ M solution: This solution was prepared by weighing 0.1165 g of indigo carmine (BDH) and dissolved in distilled water using a 250 ml volumetric flask.

Hydrochloric acid solution, 1N: An appropriate dilution of concentrated hydrochloric acid with distilled water in a final volume of 250 mL using volumetric flask to prepare 1N of acid solution.

#### 2.3. Pharmaceutical dosages:

Multivitamins Tablets*: (Multivitamin orange tablets –Germany (T&D Pharma GmbH), 10 tablets of multivitamins have been weighed, crushed, then, weighed one tablet and dissolved in a suitable volume of distilled water and stirred until the material was dissolved completely in solution. Then filtered transferred into a100.0 mL volumetric flask.

Multivitamins Capsules**: (Vitamin B-complex Capsules –India (Brawn laboratory Ltd.), ten capsules of dosage form were emptied quantitatively, mixed and weighed, then, an average weight of one capsule was dissolved with distilled water and treated similar to tablet preparation.

*Multivitamin orange tablets –Germany (T&D Pharma GmbH) AM (18), vitamin C (60), vitamin E (10), vitamin B₃(6), vitamin B₂(2) vitamin B₆(1.6), vitamin B₇(1.4), folic acid (0.2), biotin (0.15),vitamin B₁₂(0.001)

**Vitamin B-complex Capsules –India (Brawn laboratory Ltd.) NAM (10), vitamin B₃(3), vitamin B₂(1) vitamin B₆(2), vitamin B₇(5),vitamin B₁₂(0.010).
3. Results and discussion

3.1. Study of optimum conditions:
Many analytical parameters which affected on the intensity of absorbance on the colored system was studied and optimum conditions have been selected.

3.2. Effect of buffer
To check the effect of pH on the absorbance intensity of colored complex, various amounts (0.1-4.0) of 1N acids (Hydrochloric acid, Sulphuric acid, Acetic acid) were added to an aliquot of solution containing 100 µg of NAM for both methods (charge transfer and oxidation-reduction methods). The addition any amount of acid were omitted for charge transfer method because of giving no useful results. While, 1 ml of 1N hydrochloric acid was selected as an optimum amount for oxidation-reduction method as shown in fig.3.

After addition of 2 ml of alizarin reagent, The pH was 5.54, therefore different buffers (Purrin and Dempsey, 1974) at pH 5.54 were prepared such as citric acid-NaOH, sodium acetate-acetic acid, Succinic acid-NaOH, KH-Phthalate-NaOH, and Tris-Buffer. From the experimental results found that all kinds of buffers were decrease the absorbance intensity of colored system so that this study was omitted from the subsequent results.

3.3. Effect of chromate ion amount
Chromate ions for oxidation-reduction method have been studied to test an optimum amount of chromate. So, various amount of chromate 0.5-3.0 mL 8.6×10⁻⁴ M were added to various amounts of NAM (50-500) µg in a final volume 25 mL for all components. 1.5 ml of chromate solution was selected as an optimum amount due to the higher value of determination coefficient (R²=0.997139) therefore, it was recommended for the subsequent experiments (Table 1).

| ml of chromate solution 8.6×10⁻⁴ M | 50  | 100 | 250 | 500 | R²    |
|-----------------------------------|-----|-----|-----|-----|-------|
| 1.0                               | 0.189 | 0.271 | 0.361 | 0.411 | 0.9634 |
| 1.5                               | 0.211 | 0.313 | 0.501 | 0.761 | 0.9975 |
| 2.0                               | 0.182 | 0.258 | 0.441 | 0.679 | 0.9912 |

Figure 3. Effect of acid added
3.4. Effect of reagents
The amount of reagents have been studied using various amount (0.5-3.0) ml of reagents and (50-500) µg of NAM for both methods, the optimum amount of first method (charge transfer method) was 2 ml of alizarin reagent 2.5×10⁻³ M (R² =0.99743), while 1 ml of 1.0×10⁻³ M indigo carmine dye for oxidation-reduction method was an optimum amount (R² =99811).

3.5. Effect of order of addition and stability period
Effect of orders was tested experimentally for the second method (oxidation-reduction method). Table 2 showed that the order (I) was the optimum as due to the highest value of absorbance as well as the stability at the selected wavelength 610 nm of the reaction mixture (Fig.4), therefore it was selected for the subsequent experiments. The first method (charge transfer method) have only one order of addition colored system measured at 526 nm.

| Reaction components | Order number | Absorbance |
|---------------------|--------------|-------------|
| NAM + Ox + A + I.C  | I            | 0.309       |
| NAM + Ox + A + I.C  | II           | 0.282       |
| NAM + A + Ox + I.C  | III          | 0.273       |

NAM =Nicotinamide, Ox=Chromate, A=Hydrochloric acid, I.C=Indigo carmine.

3.6. Absorption spectra
NAM was treated as the suggested two spectrophotometric method (charge transfer reaction for first method and oxidation reduction reaction for second method), the spectrum and calibration curve were shown in Fig.5 and 6. Charge transfer and oxidation reduction methods have been obeyed Beer's law at 0.4-32 and 1.6-40 µg respectively in a final volume of 25 mL. Also, molar absorptivity and Sandal sensitivity were 1.95×10⁴ and 2.16×10⁴mol⁻¹.cm⁻¹, 0.00626 and 0.00565 µg⁻².cm⁻¹ for charge transfer and oxidation reduction methods respectively (Figure 7). These two spectrophotometric methods have been applied for determination of NAM in pharmaceutical dosage forms with good recoveries.
Figure 5: Absorption spectra of 100 μg of NAM /25 ml for charge transfer method measured against (A) reagent blank, (B) blank against distilled water.

Figure 6: Absorption spectra of 100 μg of NAM /25 ml for oxidation reduction method measured against (A) reagent blank, (B) distilled water, (C) blank against distilled water.

Figure 7: Calibration graph of NAM in both methods.

\[
y = 0.0064x + 0.1534 \\
R^2 = 0.9971
\]

\[
y = 0.0071x + 0.1228 \\
R^2 = 0.9985
\]
3.7. Accuracy and precision

The accuracy and precision of present methods for calibration curve were checked for determination of NAM at three concentrations, the results in table 3 show that the accuracy as well as precision were reliable.

| Amount of NAM taken, µg/25ml | Recovery*, % | RSD*, % |
|------------------------------|--------------|---------|
| 100                          | 99.31        | ± 0.276 |
| 400                          | 99.73        | ± 0.241 |
| 600                          | 100.13       | ± 0.187 |

*Average of five determinations.

3.8. Nature of the reactions

The continuous variations method (Job's method) has been used to study the reaction ratio of NAM and alizarin at the first method (charge transfer method), as well as with chromate at second method (oxidation reduction reaction), so that, the obtained results as shown in fig. 8 indicate that the ratios of NAM to alizarin and NAM to chromate was 1:1.

As a result the following reaction is suggested for charge transfer method (Figure 9) [13]:

\[
\text{Alizarine} + \text{Nicotinamide} \xrightarrow{\text{pH = 5.51}} \text{Red Complex}
\]

\[
\text{Figure 8: Job's plot for ATN - chromate}
\]

So, the reaction for oxidation-reduction method was suggested as below (Figure 10) [14]:

\[
\text{Nicotinamide} + \text{CrO}_4^{2-} \xrightarrow{H^+} \text{Nicotinamide} + \text{Cr}^{3+} + \text{H}_2\text{O}
\]

\[
\text{Figure 10: Job's plot for ATN - chromate}
\]
3.9. Effect of interferences

The selectivity and efficiency of the proposed methods was checked towards some foreign components such as (glucose, lactose, acacia, starch and menthol) that are usually founded in dosage forms. The addition of various foreign substances to 100 μg NAM in a final volume of 25 ml was studied and observed that the studied foreign materials has not interfering in the two suggested methods (Table 4).

Table 4. Effect of interferences

| Interferences | Recovery(%) of 100 μg NAM / μg of interference added |
|---------------|-------------------------------------------------------|
|               | 100         | 300         | 500         |
| Starch        | 99.64       | 99.91       | 99.93       |
| Lactose       | 99.89       | 99.82       | 99.89       |
| Glucose       | 99.71       | 99.76       | 99.78       |
| Menthol       | 100.39      | 100.72      | 100.24      |
| Acacia        | 100.36      | 100.59      | 100.34      |

Oxidation-reduction method

| Interferences | Recovery(%) of 100 μg NAM / μg of interference added |
|---------------|-------------------------------------------------------|
|               | 100         | 300         | 500         |
| Starch        | 100.34      | 100.39      | 100.48      |
| Lactose       | 99.34       | 99.45       | 99.67       |
| Glucose       | 99.89       | 99.82       | 99.79       |
| Menthol       | 99.91       | 99.89       | 99.92       |
| Acacia        | 100.42      | 100.62      | 100.45      |

3.10. Application of the method

The proposed method was successfully applied to the determination of NAM in its pharmaceutical preparation (tablet and capsule). The results which are shown in Table 5 indicate that good recoveries were obtained.

Table 5. Analytical applications

| Amount of NAM, μg | Recovery(%) of NAM* |
|------------------|---------------------|
|                  | Multivitamin orange tablets – Germany (T&D Pharma GmbH) | Vitamin B-complex Capsules – India (Brawn laboratory Ltd.) |
| 100              | 99.75               | 97.62               |
| 300              | 99.59               | 98.43               |
| 500              | 99.27               | 97.69               |

* Average of five determinations.

To calculate t-test values [15], the proposed method has been compared with literature methods [7]. Table 6 showed that t-test did not exceed the theoretical values at the 95% confidence level for five degrees of freedom when it compare with literature methods.

Table 6. The results of t-test analysis

| Drug                                | Pharmaceutical preparation | t-test | Tabulated t-test |
|-------------------------------------|----------------------------|--------|-----------------|
| Vitamin B-complex Capsules – India (Brawn laboratory Ltd.) | Capsules          | 1.605  |                 |
| Multivitamin orange tablets – Germany (T&D Pharma GmbH)     | Tablets            | 1.425  | 2.571           |
3.11. Comparison of the methods

The comparison between present methods with other literature spectrophotometric methods was shown in Table 7 which indicate that the proposed methods is sensitive and can be applied successfully to the determination of NAM in pharmaceutical preparation (Tablet and Capsule).

| Analytical parameters | Present method | Literature method |
|-----------------------|----------------|------------------|
|                       | First method  | Second method    | [7]               |
| Reaction              | Charge transfer | Oxidation-reduction | potassium iodide and potassium iodate |
| λ<sub>max </sub> (nm) | 526            | 610              | 350               |
| Reagent               | Alizarin       | Indigo-carmine   | Tri iodide ions   |
| Beer’s law range (µg/ml) | 0.4-32  | 1.6-40           | 2.5-20.0          |
| Molar absorptivity (L.mol<sup>-1</sup>.cm<sup>-1</sup>) | 1.95×10<sup>4</sup> | 2.16×10<sup>4</sup> | 0.89×10<sup>4</sup> |
| Reaction time (min)   | 90             | 90               | 610               |
| Color of the product  | Red            | Blue             | Yellow            |
| Sandell’s sensitivity (µg.cm<sup>-2</sup>) | 0.00626 | 0.00565         | 0.028             |
| R.S.D. (%)            | ±0.14          | ±0.21            | ±0.16             |

4. Conclusion

Two spectrophotometric methods were proposed for the determination of nicotinamide in its pure form as well as in its pharmaceutical preparations. These methods were rather sensitive, simple, fast, economical, and with good accuracy. The analytical parameters and statistical comparison justify this method for application the proposed method for the determination of nicotinamide in both pure and dose images. Also, the procedure does not include any tedious sample preparation or critical reaction conditions. The recommended methods have been very suitable for the determination of nicotinamide in pharmaceuticals.

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