A New Spectrophotometric Method to Determine Vitamin B6 in Pharmaceutical Formation Samples Using a Micelle Form

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Abstract
An economic and sensitive method was developed to measure pyridoxine hydrochloride (vitamin B6) in pharmaceutical formation as an ion pair, as depending on the charge transfer reaction with SDS as a surfactant and a suitable analytical reagent (chlorazol black). The parameters that gave optimum reaction conditions, such as the concentrations of chlorozal black, SDS, pH, equilibration temperature, time and effect of salting were studied to obtain a linear calibration curve where the linearity range was found to lie between 1.22×10⁻³ to 34×10⁻² mM, and the detection limit (LOD) 2.56×10⁻⁴ mM. The method was applied successfully to determine vitamin B6 concentrations in various pharmaceutical samples.

Keywords: Vitamin B6, micelle, charge transfer complex, spectrophotometric method

1. Introduction
Vitamins are biologically active organic materials with a varied chemical nature. They are generally absorbed into the human body in small quantity via food, and have a great role as biocatalysts in various metabolic processes. For each of a lack or an excess of vitamins in an organism that may be reason for a significant disturbances to many bodily functions, which can result in serious disease [1]. The vitamins of the B6 group are species that contain the pyridine ring, and are water soluble. There are six forms of Pyridoxine hydrochloride (vitamin B6) [2]. Pyridoxine hydrochloride is a crystalline white powder which is soluble in water, and a little soluble in alcohol. It melts and decomposes at about 205°C, and has a molecular structure of C8H11NO3.HCl, 205.6 g/mol [3]. Pyridoxine functions as a coenzyme in the metabolism of almost amino acids, proteins and the protection of body cells [4].

There are various analytical procedures by which to assay pyridoxine, the most significant of which include flow injection systems [5, 6] and high performance liquid chromatography for the determination of pyridoxine and isoniazid in pharmaceutical formulations [7] and in flavoured milk mixes [8], respectively, and thin layer chromatography [9]. Other methods such as a capillary electrophoresis have been developed that allow the separation and estimation of melatonin and pyridoxine in pharmaceutical preparations using electrochemical detection [10], and voltammetry [11] and cyclic voltammetry [12] have also been proposed. Many spectrophotometric methods were used to determine the pyridoxine hydrochloride; these methods include first derivative spectrophotometry to estimate pyridoxine hydrochloride combined with other drugs in tablets [13], and colorimetric methods based on the oxidation-reduction reaction of vitamin B6 with cerium (IV) ion have also been described to determine vitamin B6 in both serum and pharmaceutical preparations [14]. Diazotized sulphanilic acid [15] has also used as a reagent to determine levels of B6 vitamins in N-cetylpyridinium chloride medium. The oxidative coupling reaction has been used to determine vitamin B6 concentrations by coupling pyridoxine with 4-aminantipyrine in the presence of ammonium persulphate at 420 nm [16], while the many articles deal with using the chromatic reagent in
spectrophotometric methods to determine the inorganic and the organic materials[17-20]. Also, the chromatic reagents were used to determine various compounds by coupling with turbidity method[21-25]. The aim of study is the determination of vitamin B6 levels in various samples depending on the charge transfer reaction in the UV-Visible region with the chlorozole black T reagent, and to pre-concentrate the colour of the ion pair product is used the SDS surfactant to form micelle.

2. Experimental Section

2.1. Instruments

A Shimadzu 160A UV-Vis spectrometer was used with a 1 cm path length quartz cuvette in the measurement of sample absorption values. Acidity was measured using a benchtop pH meter.

2.2. Procedure

A solution containing 50 mgL⁻¹ of Vitamin B6 a 2 mL was added to 10 mL of 0.216 M Cu (II) resulted from that a colourless complex after that a 5 mL of 3 × 10⁻⁴ M chlorozol black solution is added to form a green colour solution have a broad peak in UV Vis spectrum, when added a 4% of SDS the colour of solution converted to pretty deep green colour because the collection the molecules by the surfactant added that form the micelles. Figure 1 shows the proposal mechanism of the reaction and the steps of the additions.
Figure 1 Proposed mechanism of the reaction that forms the micelle solution\cite{26,27}.

2.3. Materials
Distilled water was used in the preparation of all chemical substances.

*Standard solution of Vitamin B6:* A stock solution (100 mg/ml) of vitamin B6, C₉H₁₁NO₃, 169.18 g/ml, was made up.

*Stock solution of chlorazol black:* A stock solution (4 × 10⁻³ mol L⁻¹) of chlorazol black C₃₄H₂₅N₉Na₂O₇S₂, 781.73 g mol⁻¹ by dissolving 0.312 g in distilled water (100 mL).

*Copper sulphate:* A stock solution (7.2 × 10⁻³ M) of Cu⁺² was prepared by dissolving 9 g CuSO₄.5H₂O (249.6 g mol⁻¹) in 50 ml of distilled water.

*Stock solution of Sodium dodecyl sulfate (SDS):* A 4% stock solution was prepared by dissolving 1.0000 g SDS in 25 ml of distilled water.

*Stock solution of Hydrochloric acid:* A 1.00 M hydrochloric acid solution of 12.4 mol L⁻¹ was prepared by pipette mL of concentrated hydrochloric acid into a 250 ml volumetric flask and diluting to the mark.

*Stock solution of Sodium hydroxide:* A 1.00 M sodium hydroxide stock solution was prepared by weighing 4.00 g NaOH then dissolving distilled water (100 ml).

2.4. Sample preparation
The procedure was adopted for commercially available ampoules containing vitamin B6 by selecting three types from different manufacturers. The names of the different suppliers and dose of vitamin B6 in each case were recorded. The sample solutions were diluted in de-ionized water followed by filtration to remove any non-dissolved residue affecting the response.

3. Results and Discussion

3.1. The absorption spectra
The absorption spectra of the hued colour shaped from coupling vitamin B6 with Cu (II) and chlorazol black within the existence of SDS, against its relating reagent clear show greatest absorption at 477 nm. Whereas organic reagent chlorazol black with Cu (II) in the existence of SDS shows maximum absorbance at max 570 nm.

3.2. Optimization of Variables
There are various parameters affected on the absorption intensity for the coloured complex has been studied and the associated reaction conditions optimized. The preliminary investigations showed that the complex formed had a maximum absorption at 570 nm compared to the reagent blank.

3.2.1. Effect of Concentration of Copper (II) Solution:
The effect of the concentration of copper ions on the absorption of the complex was examined (for maximum colour intensity), that various from 0.072 to 0.720 mM this range appropriated with the amount of vitamin B which is added. The maximum a rich phase was achieved at 0.216 mM copper ion solution, as reported in Table 1 and illustrated in Figure 2. Accordingly, a 0.216 mM copper ion solution was used in subsequent experiments.

| Concentration of Cu(II)/ mM | Absorption measurement (n = 3) | RSD%  | Confidence interval at (95%) | Confidence interval at (95%) |
|-----------------------------|-------------------------------|-------|-----------------------------|-----------------------------|
| 0.072                       | 0.075                        | 0.773 | 0.075 ± 0.00048             | 0.075 ± 0.00048             |
| 0.216                       | 0.082                        | 0.701 | 0.082 ± 0.00048             | 0.082 ± 0.00048             |
3.2.2. Effects of the concentration chlorazol black reagent

The effects of the concentration of chlorazol black reagent on maximum formation of the coloured complex was investigated, the results of which are reported in Table 2. From the results, it was found that a 0.009 mM of chlorozal black reagent solution resulted in the greatest absorption, and therefore this concentration was selected for subsequent experiments.

Table 2 Effect of chlorazol black reagent concentration on absorption measurements [Conditions: 50 ppm of Vitamin B₆; 216 mM Cu (II); 0.1% w/v SDS]

| Concentration of chlorozol black (mM) | Absorption measurement (n = 3) | RSD% | Confidence interval at (95%) |
|--------------------------------------|-------------------------------|------|----------------------------|
| 0.003                                | 0.0523                        | 1.1032 | 0.0523 ± 0.000482          |
| 0.009                                | 0.0747                        | 0.7732 | 0.0747 ± 0.000482          |
| 0.015                                | 0.0840                        | 1.1905 | 0.0840 ± 0.000836          |
| 0.021                                | 0.0837                        | 0.6901 | 0.0837 ± 0.000482          |
| 0.030                                | 0.0837                        | 0.6901 | 0.0837 ± 0.000482          |

3.2.3. Effect of Surfactants

A surfactant, also called a surface-active agent, reduces surface tension when added to a liquid, thereby increasing the liquids spreading and wetting properties [26]. SDS is selected because of its trade availability in a high-purity homogeneous form, low cloud point temperature, slight toxicity, and low cost and the high density of the surfactant-rich phase make easy phase [28].

Table 3 Effect of SDS concentration on absorption measurements [Conditions: 50 ppm of Vitamin B₆; 0.216 mM Cu (II); 0.015 mM of chlorazol black ]

| Concentration of SDS% | Absorption measurement (n = 3) | RSD% |
|-----------------------|-------------------------------|------|
| 0.04                  | 0.0473                        | 0.0473 |
| 0.08                  | 0.0687                        | 0.0687 |
| 0.12                  | 0.0787                        | 0.0787 |
| 0.2                   | 0.0927                        | 0.0927 |
| 0.28                  | 0.0903                        | 0.0903 |
| 0.4                   | 0.0827                        | 0.0827 |

The effect of surfactant concentration on the absorbance of the complex solution due to SDS was examined within a concentration range of 0.04 to 0.4% (w/v). Table 3 and Figure 2 show that absorbance increases with increasing concentration of the surfactant, and then decreases when SDS concentration was greater than 0.2% (w/v) SDS. Therefore, this concentration is used to achieve the highest determination value.
3.2.4. Effect of pH
pH plays a critical role in metallic complex formation. A set of similar experiments was carried out in the pH range of 2.00–12.00 using different pH buffer solutions (0.1 M HCl and 0.1 M NaOH). As shown in Figure 3, the absorption first increased sharply with increasing pH, reaching a maximum at pH 7 that indicated that maximum extraction efficiency had been achieved. The absorbance values gradually decreased at higher pH due to partial dissociation of the complex which resulted in its incomplete determination. Therefore, pH 7 was chosen as the optimum working pH for complete formation of complex.

3.2.5. Effect of Temperature
The equilibration temperature in the range 10-90°C was studied. It was found that a temperature of 40°C allowed complete extraction and formation of the suspended complex to be achieved, also when increasing temperature larger than 40°C will cause complex colour change and decreasing the values of absorbance that may be because the complex is decomposition and distracted at high temperature. The effect of temperature on the formation of the complex as determined by absorption measurements is shown in Figure 4.
Figure 4 The effect of temperature on absorption [Conditions: 50 ppm of Vitamin B₆; 0.216 mM Cu (II); 0.015 mM chlorazol black; (w/v) 0.2% w/v SDS at pH 7]

3.3. Calibration Curve:
Under optimal experimental conditions, a series of standard solutions for the photometric analysis was derived from Beer’s law using very dilute solutions. The absorbance was linear over the range 1.22×10⁻³ - 34×10⁻² mM at 477 nm. The calibration graph is shown the limit of the linearity as given in Figure 5. The molar absorption coefficient was found to be 0.934 x 10³ L mol⁻¹ cm⁻¹ for copper (II). The optimal analytical parameters obtained from the above optimization experiments are summarized in Table 4.

Table 4 the parameters of the calibration curve

| Range of scatter line (mM) | Slope L mmol⁻¹ cm⁻¹ | LOD (mmol. L⁻¹) | LOQ (mmol. L⁻¹) | Intercept | r | r² | t calculated |
|---------------------------|---------------------|-----------------|-----------------|------------|---|----|--------------|
| 1.22×10⁻³ - 34×10⁻²      | 0.934×10³           | 2.56×10⁻⁴       | 8.53×10⁻⁴       | 0.0231     | 0.996 | 0.992 | 132.25       |

t critical value at 95% was 2.365

Figure 5 Scatter line for vitamin B6 determination under previously determined optimum conditions
3.4. Repeatability
The relative standard deviation (RSD%) value for Vitamin B6 is tabulated in Table 5. A RSD% of less than 3% was obtained, indicating a consistent measurement can be achieved using the proposed method.

| [Vitamin B6] mM | Average response (n = 5) | RSD% | Confidence interval at 95% |
|-----------------|--------------------------|------|---------------------------|
| 8.7, 8.7, 8.9, 8.3 and 8.5 | 8.56 ± 0.228 | 2.66% | 8.56 ± 0.09 |

Where n is the number of repeat measurements, and t critical for (n-1) at 95% was 2.132

3.5. Application of the Proposed Method
To test the applicability of the method devised above, it was applied in the determination of the amounts of Vitamin B6 in pharmaceutical preparations. On applying the proposed procedure, good recovery was obtained, as reported in Table 6.

| Name of Drug | Theoretical Value of Vitamin B6 mmol.L⁻¹ | Measured value of Vitamin B6 mmol.L⁻¹ | Recovery % |
|--------------|----------------------------------------|--------------------------------------|------------|
| Becozyme, BAYER(France) (4 mg) | 9.7 | 8.6 (n = 5) | 88.56% |
| ANCOPIR, Moskhimfarmpreparaty (Turkey),(100 mg) | 0.121 | 0.126 (n = 5) | 104.13% |
| Neurorubine, Acino (Switzerland) (100 mg) | 48.6 | 48.0 (n = 5) | 98.76% |

n: no. of repeat measurements

4. Conclusion
A simple, rapid, accurate and precise spectrophotometric method was evaluated in terms of its ability to determine concentrations of pyridoxine (Vitamin B6) in both pure and pharmaceutical formulations. The little analyses time and economic cost are the main advantages of this method for the purposes of routine quantity analysis.

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