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A Spectrophotometric Approach for Estimating Nimodipine by Oxidative-Coupling Reaction with 4-Aminoantipyrine in its Tablet and Biological Fluids

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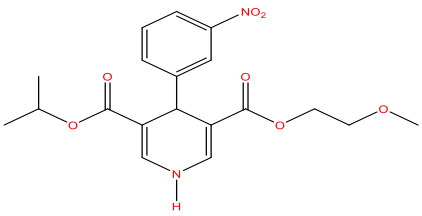
Abstract

This study involves the development of a speedy, artless, selective and precise spectrophotometric methodology for the determination of nimodipine in the aqueous medium. The proposed approach was dependent on the oxidation and coupling reaction of nimodipine with 4-aminoantipyrine using KIO3 as an oxidizing agent. A yellow-brown product was fashioned at room temperature which gave a band with maximum absorption at 464 nm. Calibration plot was linear and adheres to Beer's law within the concentration range 1.0-35 µg/ml with an excellent coefficient of determination (R2=0.9992). The values of molar absorptivity and Sandell's sensitivity were planned and established to be in the consequence of 1.2679x104 l/mol.cm. and 0.033 µg/cm2, correspondingly. The mole ratio of the achieved product has been measured between nimodipine and 4-aminoantipyrine was evaluated to be 1:1. The detection limit (LOD) and quantitation limit (LOQ) were expected and found to be 0.01774 and 0.05914 µg/ml, correspondingly. The recoveries percentage were obtained in the range 97.38%-103.61% while, the precision (RSD) was in the range 0.96%-3.18% depending on the concentration level. The recommended approach was applied successfully to estimate nimodipine in its tablet and in the biological fluids, no intrusions were noticed from the shared excipients additives present in the commercial pharmaceutical formulation.

Keywords: Nimodipine, 4-Aminoantipyrine, Potassium iodate, Oxidative-coupling, Spectrophotometry.

Introduction

Nimodipine (NDP) is a 1,4-dihydropyridine calcium channel blocker that acts by relaxing the arterial smooth muscle [1]. NDP is famous for its favoured action on cerebral vessels than other agents within the same class [2]. It's work depends on relaxing the muscles of blood vessels, especially in the brain, other uses is to treat senile dementia and to prevent the vasospasm [3]. The IUPAC name of NDP is 3,5-pyridinedicarboxylic acid,1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-,2-methoxyethyl 1-methylethyle ester. The molecular formula of NDP is $C_{21}H_{26}N_2O_7$ and its molecular weight 418.44 g/mol (Scheme 1) [4].



Scheme 1: Chemical structure of NDP.

A number of analytical procedures have been described in the literatures for the determination of NDP in different subjects like bulk, pharmaceutical forms and biological fluids. These techniques included, RP-HPLC [5], HPLC-UV [6,7], liquid chromatography-tandem mass spectrometry [8], Mass spectrometry conjugated with liquid chromatography [9] spectrofluorometry [10], HPLC-electrospray ionization-mass spectrometric) [11], differential pulse voltammetry using modified reduced graphene oxide composite [12], square wave voltammetry (SWV) [13], cathodic adsorptive stripping voltammetry [14], and atomic absorption spectroscopy [15]. Most of these methods were required critical working conditions, heating step and an expensive equipment and a skilled operation.

Spectrophotometric methods still utilized widly due to its economic, sensitivity, selectivity, and rapidity. Therefore, different UV-visible spectrophotometric methods were used for determining NDP in aqueous medium. Some of these approaches included diazotization of the reduced NDP and coupling with phloroglucinol [16], acetylacetone, diphenylamine, citrazinic acid and chromotropic acid [17], resorcinol [17]. Others based on the ion-pair extraction-spectrophotometry [4], using tetrabutylammonium hydroxide reagent to produce yellow colour product [18], condensation reaction with p-anisaldehyde [19], coupling reaction of NDP with vanillin reagent in acidic medium [20], oxidation of NDP with potassium permanganate [21], charge transfer complex reaction [22] and bleaching the colour of indigo carmine dye using N-bromosuccinimide as oxidant [23]. UV-spectrometric methods [24,25], have also been used for determining NDP. However, majority of these methods suffer from various difficulties, for instance, low range of estimation, poor selectivity and moderate sensitivity. Others were typically time consuming, costly chromogenic reagent, required organic-solvent extraction or heating conditions. The focal aim of the present approach is to develop more advantageous spectrophotometric technique for assaying NDP in bulk sample and in its tablet and biological fluids.

Experimental

Equipments

A digital double beam UV-Vis spectrophotometer device (Jasco V-630) was equipped with 1 cm matched fused silica cells was used for recording the absorption spectra and recording the absorbance values. A pH meter (Bp3001 professional bench top) was also employed for recording the values of pH readings.

The Sample of Drug

Pure NDP drug was equipped from KOMJEI Technol Chunangilian Building Road 2 Qianjin Bao An Shenzhen CHINA and used as gift.

Reagents and Materials

All chemical materials and reagents were of analytical grade.

Standard reduced NDP solution 100 (µg/mL) ($2.3898x10^{-4}$)M: A 10 mg of pure NDP was weighed exactly, transferred into 100 mL beaker and dissolved in 5 mL of methanol. A 5mL of 4N hydrochloric acid and 1g of zing powder were added. The solution was shaken thoroughly for about 20 min. The solution was then transferred quantitatively to a 100 ml standard flask and adjusted up to the mark with distilled water to give a standard solution of 100 µg/mL of NDP. The filtration is accomplished using Whitman No.4 filter paper [26].

4-Aminoantipyrine Solution (0.1%): This solution is prepared by weighing 0.1 g of 4-aminoantipyrine (4-AAP) and dissolved in 3 mL of methanol, the solution was then transferred to a 100 mL standard flask, and diluted to the mark with distilled water.

Potassium Iodate Solution (0.2%): A 0.2000 g of potassium iodate (KIO_3) was dissolved in a little amount of hot distilled water and shacked well for a few minutes. The solution was then transferred to a 100 mL standard flask and the volume was completed to the mark using the same solvent.

Sodium Hydroxide Solution (0.1M): It was prepared by diluting appropriate volume of the standard sodium hydroxide solution (1M) to a 100-mL with dw using standard flask and the prepared solution was then stored in a plastic bottle.

General Procedure

An aliquot containing an increasing concentrations 1.0-35 µg/ml of reduced standard NDP solution was transferred quantitatively to a series of 10 mL standard flasks. To each, a 1ml of potassium iodate (0.2%) was added, waited for 5 minutes to complete the oxidation of the drug, and then followed by 2 mL of 4-aminoantipyrine (0.1%) and 0.7ml of sodium hydroxide 0.1m. The flasks were mixed thoroughly and the solutions were diluted to the mark with dw. After that the absorbance was measured at 464 nm versus the corresponding reagent blank.

Preparation of Pharmaceutical Tablet Solution

Ten tablet of nimotap each one contains 30 mg of NDP were weighed exactly and ground into a fine powder. An equivalent to 10 mg of pure NDP was accurately weighed and dissolved in 5 mL of methanol using a conical flask, followed by 5 mL of 4N HCl and 1.0 g of zinc dust. The contents were mixed continuously for about 20 minutes and transferred quantitatively to a 100-mL standard flask and the volume was completed to the mark with dw. The solution was then filtered through Whitman No.41 filter paper. An aliquot of the tablet solution was taken and the amount of NDP was analysed according to the recommended procedure.

Procedure for Spiked Biological Fluids

Serum and urine samples were delivered from some healthy volunteers [27,28].

To a 1 mL of serum sample, 5 mL of acetonitrile was added and mixed well. The sample solution was then placed in a centrifuge for 5 minutes at 2500 rpm. The supernatant was used for examining the NDP recovery percentage. While for urine, A 1 mL of spiked urine sample was 50-fold diluted with dw .

For both samples, an appropriate amounts of reduced NDP standard solution (100 µg/mL) were added to 0.5 and 1 mL of the treated serum and urine and the concentration of NDP was estimated by following the recommended procedure.

Results and Discussion

Principle of the Method

The procedure depends on the oxidative coupling reaction of NDP and 4-aminoantipyrine reagent in presence of potassium iodate in a basic medium to form a product with an intensely yellow-brown colour.

Optimization of the Reactions, Conditions

All the different parameters that affected on the sensitivity of the yellow-brown colour product have been accomplished using 10 µg/mL NDP, 1 mL of 4-aminoantipyrine solution, 1 mL of alkaline solution and 10 ml of standard flask.

Oxidizing Agent Effect

The influence of 1 mL of diverse oxidizing compounds (0.2%) on the absorbance of the resulting product have been investigated. The results in Table (1) show that the reaction of NDP with 4-aminoantipyrine in the existence of potassium iodate is the superlative, because potassium iodate gives the highest sensitivity and a good colour contrast. Therefore, it has been selected for the next investigations.

Oxidizing agent (0.2%)	Absorbance	λ_{max} (nm)
N-Bromosuccinamide	0.0315	384
N-Chlorosuccineamide	0.0458	386
Sodium periodate	0.0417	501
Potassium periodate	0.3133	342
Potassium iodate	**0.2380**	464
Chloramine-T	0.0052	382

Table 1: Effect of oxidizing agent on absorbance.

Optimization of Potassium Iodate Concentration and its Volume

The influence of altered concentrations 0.15- 0.35% of potassium iodate was tested on the absorbance of the resulting colour product. The results in Table (2) reveal that the concentration 0.2% of potassium iodate is the optimal, therefore, it has been chosen for the subsequent studies.

KIO_3 conc. (%)	0.15	0.17	0.2	0.25	0.3	0.35
Absorbance	0.1935	0.2295	**0.2383**	0.2350	0.2281	0.2257

Table 2: Concentration influence of KIO_3 on absorbance.

The influence of diverse volumes 0.3-2.0 mL of potassium iodate on the absorbance of NDP was studied. The experimental results in Table (3) explain that the greatest absorbance value was obtained when using 1 mL of 0.2% potassium iodate (A=0.2385), therefore it has been dependent for the subsequent studies.

mL of (0.2%) KIO_3	Absorbance
0.3	0.0260
0.7	0.1674
1.0	**0.2385**
1.5	0.2118
2.0	0.2103

Table 3: Influence of KIO_3 amount.

Influence of the Volume of 4-Aminoantipyrine Solution

The colour of resulting product was found dependent on the amount of 4-aminoantipyrine reagent and it was observed that the absorbance values increased as the amount of 4-aminoantipyrine was increased. So, the influence of several quantities from 1 to 3 mL of 4-aminoantipyrine reagent (0.1%) on absorption was examined. Table (4) shows that the absorbance reaches the optimum on using 2 mL of 4-aminoantipyrine which gives an excellent determination coefficient (R2=0.9992). Thus, the using of 2 mL of 4-aminoantipyrine reagent was recommended for the subsequent experiments.

mL of 4-AAP (0.1%)	Absorbance/µg of NDP added					R^2
	25	50	75	100	150	
1.0	0.0432	0.1172	0.1895	0.2386	0.4125	0.9936
1.5	0.0652	0.1564	0.2156	0.3161	0.4701	0.9970
2.0	0.0832	0.1669	0.2720	**0.3625**	0.5407	**0.9992**
2.5	0.0660	0.1325	0.2478	0.2673	0.4201	0.9961
3.0	0.0583	0.1209	0.1876	0.2416	0.3935	0.9968

Table 4: Influence of 4-aminoantipyrine reagent amount on absorbance.

Temperature and Oxidation Time

The influence of different four temperature 5, LT, 50 and 70Cº using a thermostatic ice and water bath at different periods of time before the addition of 4-aminoantipyrine reagent on the absorption intensity of the resulting product has been investigated. The results in Figure (1) confirmed that the oxidation of NDP with potassium iodate was completed after 3 minutes at laboratory temperature (LT.=25±2 Cº).

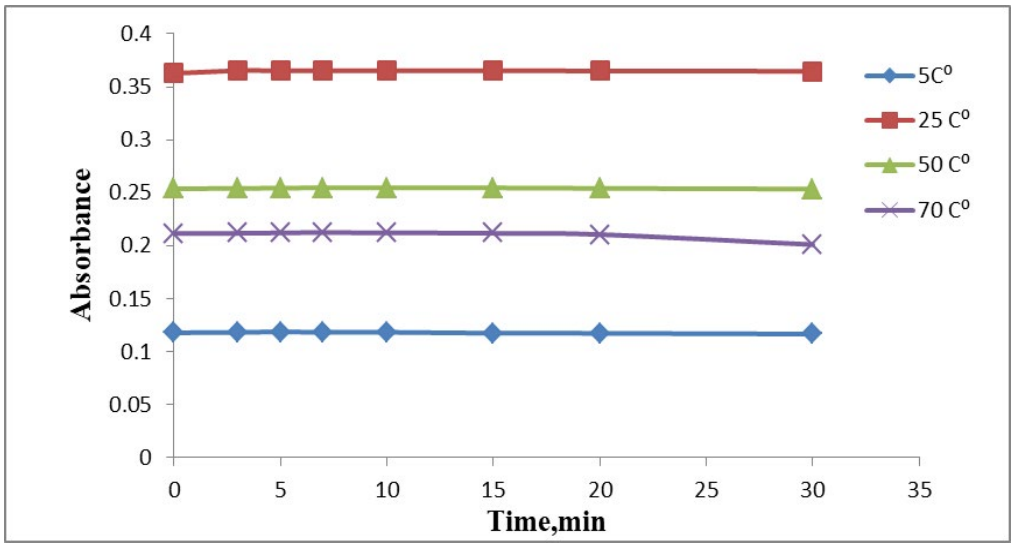


Figure 1: Effect of temperature and oxidation time on absorbance.

Influence of the Basicity Type and its Amount

From the essential experiments, the oxidative-coupling reaction occurred in an alkaline medium, therefore the influence of different quantities 0.3 to 2.0 mL of 0.1M various bases (NaOH, KOH, Na_2CO_3, $NaHCO_3$ and NH_4OH) on absorbance of the colour product have been reviewed. Maximum absorbance with a good sensitivity was achieved when the reaction carried out by using 0.7 mL of sodium hydroxide (0.1M), the results are shown in Figure (2).

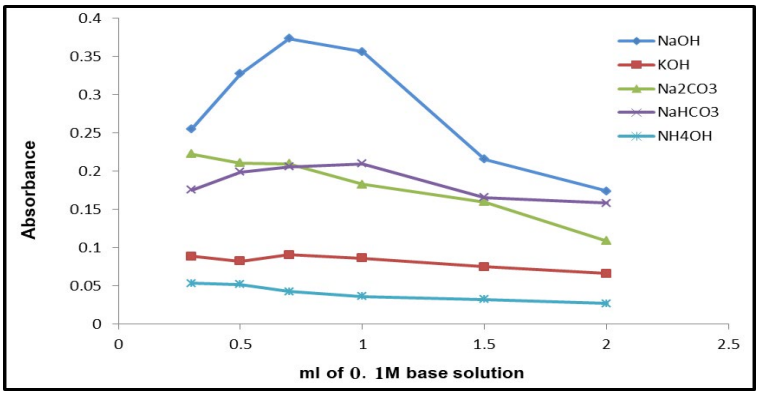


Figure 2: Influence of base and its quantity on absorbance.

Effect of Surfactants

This effect was checked by adding various types of surfactant to the contents of the reaction mixture such as, sodium dodecyl sulphate (SDS), cetylperdinum chloride (CPC), and triton x-100 as anionic, cationic and neutral surfactant, respectively. It was observed practically the using of all these surfactants caused no effect on the sensitivity (they decreased the absorbance values) of the resulting product, therefore, the addition of them was neglected from the subsequent studies.

Influence of Addition Order

The influence of changing the sequence addition of reactants on the colour development was studied. The best order which gave the maximum absorbance (A=0.3735) is (NDP+ potassium iodate + 4-aminoantipyrine + NaOH) and is adopted for the subsequent studies.

Time effect on Colour Development

Beneath the optimal reaction conditions, the influence of time on the colour development of the resulting product was tested by measuring the absorbance of the coloured product at different intervals. The results are shortened in Figure (3) indicated that the colour was produced immediately at laboratory temperature and there is no noticeable change is appeared on absorbance for more than 60 minutes.

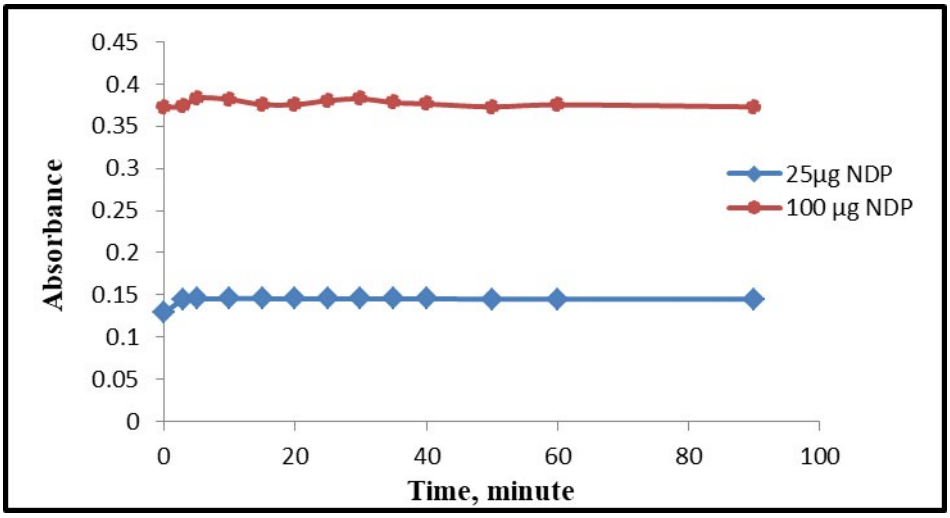


Figure 3: Effect of time on stability of the product.

Summary of Optimal Conditions and Final Absorption Spectra

After optimizing all the reaction conditions which approved for estimating NDP are exemplified in Table (5). The final absorption spectrum of the resulting yellow-brown product was obtained from the reaction of NDP with 4-aminoantipyrine reagent using potassium iodate as an oxidant in an alkaline medium. Figure (4) clarify that the product exhibits a maximum absorption peak at 464 nm against corresponding reagent blank.

Variables	Optimality
λ_{max} (nm)	464
Type of oxidant ,concentration, mL	KIO_3, 0.2%, 1
Type of reagent, concentration, mL	4-Aminoantipyrine, 0.1%, 2
Type of base, concentration, mL	NaOH, 0.1M, 0.7
Oxidation time (minute)	3
Temperature (°C)	Laboratory temperature
Stability period (minute)	≥60

Table 5: Summary of the optimal conditions.

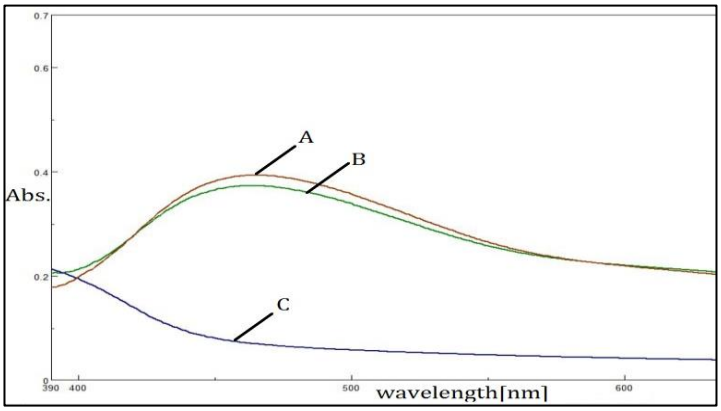


Figure 4: Final absorption spectra of 10 µg/mL of NDP measured Vs. (A) blank solution, (B) dw and (C) blank solution Vs. dw.

Linearity of the Calibration Graph, Quantification and Detection Limits

Beneath the specified optimum conditions a linear calibration graph was obtained by plotting the absorbance values against concentration of NDP. The resulting product adheres to Beer's law in the concentration range from 1.0 to 35 µg/ml NDP with an excellent determination coefficient (R^2) equal to 0.9992 (Figure (5)). The Sandell's sensitivity, molar absorptivity, LOD and LOQ were valued and found to be 0.033 µg/cm^2, 1.2679×10^4 l/mol.cm, 0.01774 and 0.05914 µg/ml , correspondingly [29].

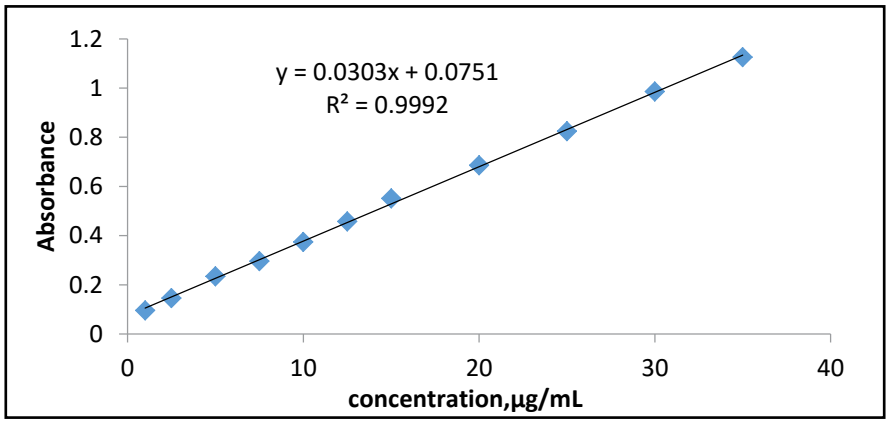
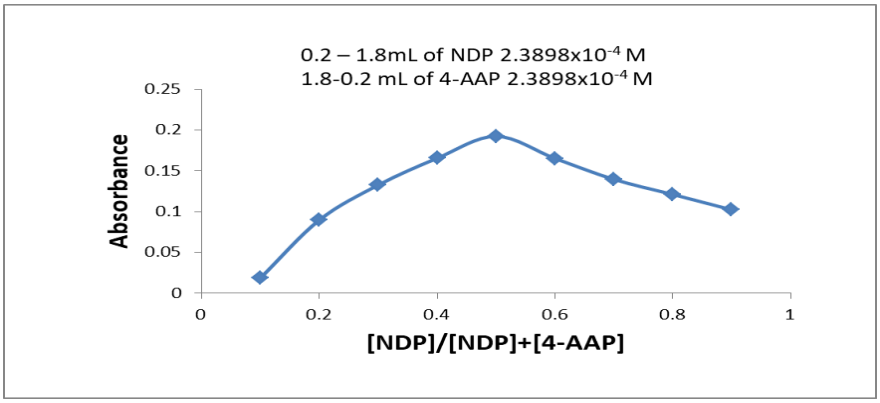


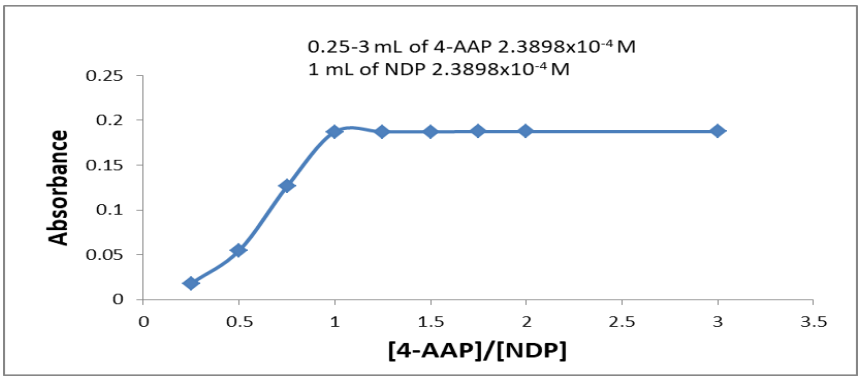
Figure 5: Calibration plot for the assaying of NDP.

Nature of the Resulting Product

Under the optimized reaction conditions of the recommended procedure, continuous variation (Jop) and mole ratio methods [30] were employed to elucidate the correlation ratio of NDP with 4-aminoantipyrine. Both methods were carried out by using the same concentrations of NDP and 4-aminoantipyrine solutions 2.3898x10^{-4} M. The results of both methods are exemplified in Figure (6) revealed that the coloured product was fashioned by a 1:1 combining ratio of NDP to 4-aminoantipyrine.



A

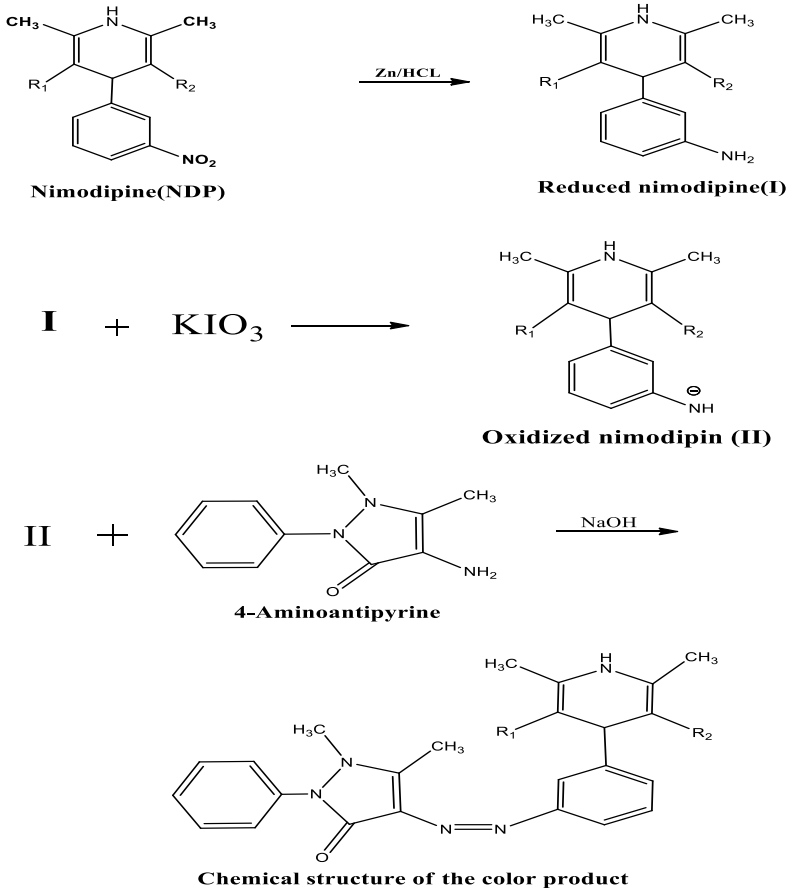


B

Figure 6: The plots of continuous variation (A) and mole ratio methods (B)

Mechanism of the Reaction

According to the results of stoichiometry investigation, the suggested reaction mechanism of the interaction of NDP with 4-aminantipyrine in presence of potassium iodate as an oxidant to give a yellow-brown color product was based on e principle of oxidative coupling reaction can be elucidated in the Scheme 2. [31].



R1 = -COO-CH-$(CH_3)_2$
R2 = -COO-CH_2-CH_2-O-CH_3

Scheme 2: Chemical structure of the resulting coloured product.

Application

The applicability of the recommended method has been applied for the determination of NDP in commercially available pharmaceutical formulation (tablet) and biological fluids (urine and serum) at three different quantities 25,100 and 200 µg of NDP. The data are listed in Table (6) revealed that the suggested method succeed for assaying NDP with an acceptable results with good recoveries.

Sample	NDP found (µg)	Recovery* (%)	R.E* (%)	RSD*(%) (N=5)
Nimotap 30mg/tablet (Germany)	24.38	97.38	-2.48	2.22
	98.63	98.63	-1.37	1.24
	197.51	98.75	-1.245	1.37
Urine**	24.67	98.68	-1.32	0.96
	103.61	103.61	3.61	1.25
	201.52	100.76	0.76	2.17
	25.39	101.56	1.56	1.58
Serum**	103.05	103.05	3.05	3.18
	203.10	101.55	1.55	1.44
*Average of five determinations ; **Volume of sample=1 Ml				

Table 6: Analysis of NDP in pharmaceutical formulation and biological fluids.

Evaluation of the Method

To evaluate the results of the recommended method a t-test has been applied. The acquisition data which listed in Table (7) reveal that the values of experimental (t) were under the artificial t-value (2.776) at 95% confidence level and for four degrees of freedom (N=4) [32]. As shown there is no significant difference between the tabulated and experimental values which refers to the possibility of using the recommended method for estimating NDP in its pharmaceutical form (tablet) and biological fluids.

Sample	mL of sample	NDP Found (µg)	Recovery* (%)	R.E.* (%)	RSD*(%) (N=5)	t-exp.**
Nimotap 30mg/tablet (Germany)	-	98.63	98.63	-1.37	1.242	1.638
Urine	0.5	96.55	96.55	-3.45	3.05	1.04
	1.0	103.52	103.61	3.61	1.25	1.32
Serum	0.5	97.86	97.86	-2.14	2.19	0.95
	1.0	103.05	103.05	3.05	3.18	1.63
*Average of five estimations; **t-exp: (t-experimental); ± t = $(\bar{X} - \mu)\frac{\sqrt{N}}{S}$; Tabulated t-value at 95% confidence level is equal to 2.776; degree of freedom (N=4)						

Table 7: Determination of NDP in tablet and biological fluids.

Validity of the Proposed Method

In order to check the validity of the suggested method, standard addition method was carried out and proved that the recommended procedure can be successfully applied for estimating NDP without interferences. The data are illustrated in Figure (7) and listed in Table (8) indicated that the standard addition method agree well with the results of the proposed method within an acceptable range of error.

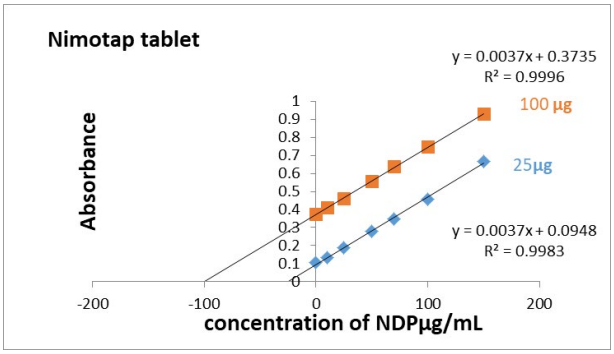


Figure 7: Standard addition method for the determination of NDP in tablet.

Drug	Certified value	NDP(µg)		Recovery(%)	R.E.(%)	Measured value (mg)
		Present	found			
Nimotap (Germany)	30 mg/ tablet	25	25.62	102.48	2.48	30.74
		100	100.94	100.94	0.94	30.28

Table 8: Analysis of NDP in commercial tablet by standard addition method.

Conclusion

This work describes a simple, accurate and sensitive spectrophotometric method for the estimation of NDP as pure form, in the pharmaceutical formulation (tablet) and biological fluids through oxidative coupling reaction. The method does not need solvent extraction steps or temperature control. The method was also precise and selective to be successfully applied for estimating NDP in various samples with good recoveries.

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