ISSN-1996-918X

Pak. J. Anal. Environ. Chem. Vol. 24, No. 2 (2023) 185 – 196

http://doi.org/10.21743/pjaec/2023.12.07

Improved Spectrophotometric Estimation of Nimodipine in the Pharmaceutical Formulation and Biological Fluids

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Received 10 April 2023, Revised 30 August 2023, Accepted 13 October 2023

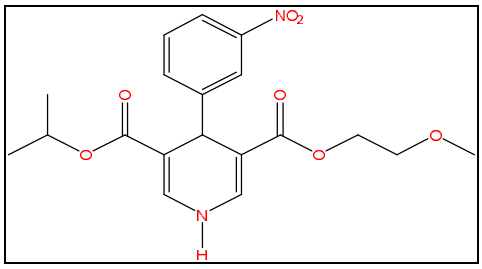
Abstract

Nimodipine (NDP) belongs to the class of pharmacological agents known as calcium channel blockers. It is used to treat symptoms resulting from a ruptured blood vessel in the brain (hemorrhage). NDP increases blood flow to injured brain tissues. For its clinical importance, a sensitive, accurate and simple spectrophotometric approach for the evaluation of NDP in bulk, tablet and biological fluids has been developed. The procedure involves the reaction of the reduced NDP with equimolar nitrous acid then followed by coupling with the available γ-resorsolic acid reagent in a basic solution to give a colored azo dye. The resulting azo dye is soluble in water and shows a maximum absorption peak at 436 nm. All the variables which affect the conditions such as the influence of acid, γ-resorsolic acid and alkali concentrations, reaction time and Beer's law limits were studied carefully and adjusted. The optimal conditions showed the color of azo-dye was stable for more than 1 h. The method was found linear in the range from 1.0 to 40 µg/mL with a good value of determination coefficient (R^2= 0.9988). The molar absorptivity was calculated and set up to be 1.8495×10^4 L/mol.cm. The detection limits and quantitation limits were also estimated and found to be 0.0059 and 0.0195 µg/mL, correspondingly. The approach was established by estimating NDP in pharmaceutical tablets and biological fluids. The precision (RSD) was calculated to be better than 0.324%, whereas the values of recovery percent and relative errors were in the range of 97.95% to 99.08 % and -3.78% to 2.22%, respectively, without interfering from any common excipients present in the samples. The nature of the yellow dye has been studied between diazotized NDP and γ-resorsolic acid reagent and was equal to 1:1.

Keywords: Nimodipine, γ-Resorsolic acid, Diazo-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 favored action on cerebral vessels over other agents within the same class [2]. It's likely cytoprotective effects by reducing calcium inflow into nerve cells [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 is 418.44 g/mol (scheme1) [4].



Scheme 1.Chemical structure of NDP.

A number of analytical techniques have been described in the literature for the determination of NDP in different subjects like bulk, pharmaceutical forms and biological fluids. These techniques included RP-HPLC [5], LC-electrospray tandem mass spectrometry [6], LC-tandem mass spectrometry [7], LC-MS/MS [8], spectrofluorometry [9], HPLC-UV [10] and [11], HPLC-ESI-MS [12], differential pulse voltammetry using modified reduced graphene oxide composite [13], square wave voltammetry (SWV) [14], cathodic adsorptive stripping voltammetry [15], UV-spectrophotometry [16] and atomic absorption spectroscopy [17]. Most of these methods required critical working conditions, heating steps, expensive equipment and skilled operation.

Spectrophotometric methods are still utilized widely due to their sensitivity, selectivity, rapidity and cost-effective. Therefore, different UV-visible spectrophotometric methods were used for determining NDP in aqueous medium. Most of these approaches involved diazotization of the reduced nimodipine and coupling with phloroglucinol [18], acetylacetone, diphenylamine, citrazinic acid and chromotropic acid [19], β-naphthol [20], resorcinol [21]. Others based on the ion-pair extraction-spectrophotometry [22], condensation reaction with p-anisaldehyde [23], coupling reaction of NDP with vanillin reagent in acidic medium [24], oxidation-reduction reaction with potassium permanganate [25], charge transfere complex formation [21], bleaching reaction using N-bromosuccinimide as oxidant and indigo carmine dye [26] and anion-formation using tetrabutylammonium hydroxide reagent (TBAH) [27]. However, the majority of these methods suffer from various difficulties, for instance, low range of estimation, poor selectivity and moderate sensitivity. Others were typically time-consuming, applicable to higher concentrations of NDP and high detection limit value or required solvent extraction. The aim of this approach is to create an economic, rapid, simple and precise spectrophotometric procedure using γ-resorsolic acid (2,6-dihydroxybenzoic acid) for assaying NDP in the bulk, pharmaceutical formulation and biological fluids.

Materials and Methods

Instrumentations

A Jasco V-630 digital double-beam UV-Vis. spectrophotometer equipped with 1.0-cm matched fused silica cells and Bp3001 professional benchtop pH meter instruments were used for all recording absorption spectra and pH measurements, respectively.

Materials and Reagents

All reagents and chemicals used were of a high degree of purity and the standard material of nimodipine was obtained from KOMJIE Technol Chuangilian Building Road 2 Qianjin Bao Shenzhen, China**.**

Standard reduced NDP solution, 100 µg/mL ($2.3898x10^{-4}$M)

An accurate 10 mg of pure NDP was weighed and dissolved in 5 mL methanol. To this solution, 5 mL of 4 N hydrochloric acid and 1.0 g of zinc dust were added and shaken thoroughly for about 20 min. The solution was then transferred quantitatively to a 100 mL calibrated flask and made up to the mark with distilled water (dw). The prepared final solution was filtrated using Whatman No. 41 filter paper [28].

Stock sodium nitrite solution (0.1%)

A 0.1 g of sodium nitrite was dissolved in 100 mL dw using a calibrated flask and stored in a dark bottle.

Working sodium nitrite solution (2.3898×10^{-4}) M

It was prepared by diluting the appropriate amount of the sodium nitrite stock solution with dw to get the desired concentration and then transferred the prepared solution into a dark bottle.

γ-resorsolic acid (0.1%)

It was prepared by dissolving 0.10 g of γ-resorsolic acid in a 100 mL dw.

Hydrochloric acid 1.0 M

A 8.5 mL of the concentrated acid (11.76 M) was pipetted into 100 mL volumetric flask and made up to the mark with dw.

Sodium hydroxide solution 1.0 M

A 4.0g of sodium hydroxide tablet was dissolved with dw using a 100 mL dw calibrated flask and the prepared solution was then stored in a plastic bottle.

Interferences solution 1000 µg/mL

A 0.10 g of common excipients such as glucose, lactose, gum arabic, sorbitol and starch were dissolved separately in a 100 mL dw.

Serial volumes of reduced NDP stock solution from 0.1 to 4.0 mL were accurately transferred to 10 mL calibrated flasks followed by the addition of the same volume of sodium nitrite working solution, which was in the same molarity concentration of NDP, to each flask 0.5 mL of 1.0 M hydrochloric acid was added. After 5.0 min at room temperature, 1.5 mL of γ-resorsolic acid (0.1%) and 1.0 mL of 1 M sodium hydroxide were added. The contents were allowed to stand for 5.0 min and the final volume was then made up to 10 mL with dw and mixed well. After 5 min the absorbance of the resulting azo-dye was measured at 436 nm against a reagent blank containing all materials except NDP.

Analysis of nimodipine in the tablets

Ten tablets of nimodipine (Germany) (each tablet containing 30 mg of NDP) were taken and crushed into fine powder using a pestle and mortar. A quantity of the powder equivalent to 10.0 mg of NDP was weighed accurately and dissolved in 5.0 mL methanol. Then, 5.0 mL of 4 N hydrochloric acid, and 1.0 g of zinc dust were added, the contents were shaken thoroughly for about 20 min and the solution was then diluted to the mark with dw. The solution was mixed well and filtered using Whatman No. 41 filter paper. A suitable amount of the drug solution was taken and the suggested standard procedure was followed to analyse the NDP in the tablet.

Analysis of nimodipine in biological fluids

Serum and urine samples were provided from several healthy volunteers. To 1.0 mL of serum, 5.0 mL of acetonitrile (absolute) was added to deproteinize. The solution was then put in a centrifuge for 5.0 min at 2500 rpm. The supernatant was used for investigating recovery. Spiked urine was 50-fold diluted with dw. A convenient amount of NDP standard solution was added to a known volume of the treated serum and urine sample solution. The analysis of NDP was followed as in the suggested procedure [29, 30].

Essential procedure

Results and Discussion

Principle of the Method

In this developed research, after reducing aromatic nitro group to an amino group. The reduced NDP was diazotized with an equimolar of sodium nitrite in an acidic

medium to produce corresponding diazonium salt. Then the resulting diazonium salt was coupled with a suitable coupling reagent in a basic medium to form an intensely yellow colored azo-dye.

Optimization of Experimental Variables

The various experimental variables affecting mainly the sensitivity, stability and formation of the colored-azo dye were optimized. The subsequent experiments were performed using 100 µg of pure NDP solution in a final volume of 10 mL of calibrated flask and the absorbance of the resulting azo dye was recorded at the suitable wavelength of λmax against its blank solution.

Choosing of Suitable Coupling Agent

Many aromatic amino and phenolic coupling agents have been checked for efficient conditions. The results presented in Table 1 show that the reagent of γ-resorsolic acid was considered the best coupling agent according to the presence of two strong electron donating (hydroxy groups), therefore it has been selected as the suitable coupling agent and all the absorbance measurements were recorded at λmax of 436 nm.

Table 1. **Effect of various coupling agents on absorbance.**

mL of coupling reagent (0.1%)	Absorbance	λmax (nm)	Δλ (nm)*
8-Hydroxyquinoline	0.0835	365	------
3,4-Dimethyl phenol	0.0248	420	45
Phenylephrine	No color contrast	------	------
γ-resorsolic acid	0.3071	436	156
Pyrogallol	0.0131	313	------
3-Hydroxy-1-methyl-2-quinone	No color contrast	------	turbid
p-Amino phenol	0.0123	380	------
p-Aminobenzophenone	No color contrast	------	------

* Δλ = λmax of sample - λmax of blank

Effect of Acid Type and Its Quantity

The acid solution is necessary for the diazotization process. Therefore, the effect of 1 mL of several acid solutions, including HCl, HNO_3, H_2SO_4, CH_3COOH, and HCOOH, on the dye's absorbance has been studied. The solution of HCl showed a satisfactory value (A=03088) than the other mineral acids (Fig. 1). The effect of different quantities of 0.1-1.5 mL of 1 M HCl on the sensitivity of the azo dye was also investigated and the results in Table 2 reveal that 0.5 mL of 1 M HCl is the optimum, therefore it was established for the next experiments.

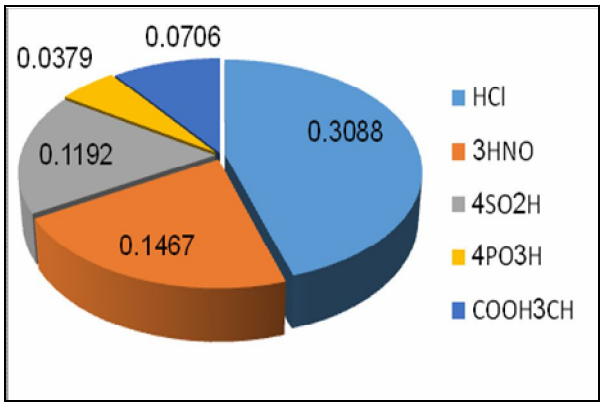


Figure 1. **Effect of 1 M various acids on the absorbance of the azo-dye**

Table 2. **Effect of HCl amount on absorbance.**

1 M HCl solution (mL)	0.1	1.5	1.0	0.7	0.5	0.3
Absorbance	0.1344	0.1152	0.3092	0.3267	0.3880	0.2864

The influence of diazotized reaction time on the azo dye's absorption intensity was carried out by measuring the absorbance at different intervals of time at laboratory temperature (25±2 °C) and the results are illustrated in Fig. 2.

The results in Fig. 2 illustrated that the absorbance reaches a maximum after waiting for 5-6 min, therefore, 5 min was selected for the subsequent investigation.

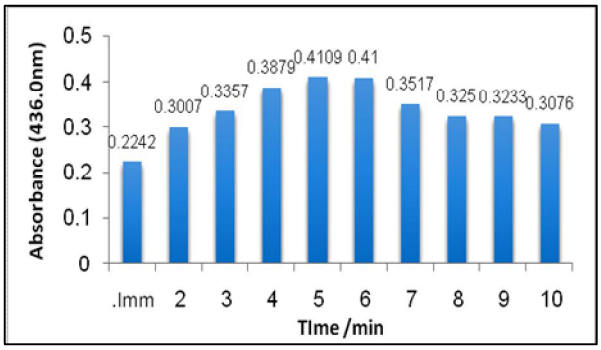


Figure 2. **Effect of diazotization reaction time on absorbance**

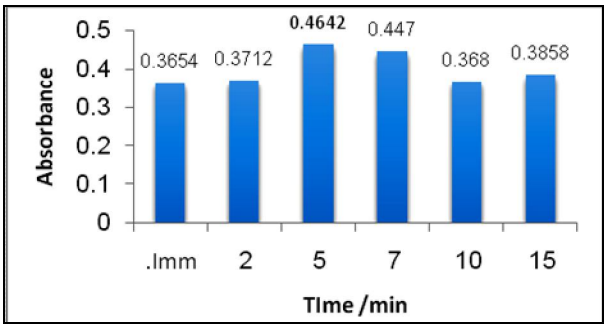


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Figure 3. **Influence of coupling reaction time on absorbance**

Influence of the Reagent Amount

The effect of diverse quantities 0.5 to 2.0 mL of γ-resorsolic acid and an increasing amounts 25, 50, 75, 100, 150 µg of NDP on the sensitivity of the method was carried out. The results are explained in Table 4.

Table 3. **Effect of γ-resorsolic acid amount.**

γ-resorsolic acid reagent (0.1%) mL	Absorbance / µg of NDP added					R^2
	25	50	75	100	150	
0.5	0.0652	0.1195	0.1815	0.2276	0.3378	0.9989
1.0	0.0982	0.1937	0.2874	0.4112	0.5620	0.9941
1.5	0.1220	0.2433	0.3546	0.4633	0.6818	0.9996
2.0	0.1218	0.2356	0.3468	0.4553	0.6512	0.9987

The results in Table 3 reveal that the volume of 1.5 mL of the reagent γ-resorsolic acid is the optimum because it shows the greatest intensity of absorption and a good value of the determination coefficient (R^2=0.9996). Therefore, 1.5 mL of γ-resorsolic acid was verified in this work.

The influence of coupling reaction time of γ-resorsolic acid on absorbance was also studied. The results in Fig.3 illustrate that the coupling of reduced NDP with γ-resorsolic acid needs at least 5-7 min to be completed and thus, 5 min was fixed in the subsequent experiments.

Influence of Base Type and its Quantity

In order to select the effective type of base for the reaction, the effect of different quantities of various aqueous bases, such as sodium, potassium and ammonium hydroxides, sodium carbonate and bicarbonate having the same concentration (1 M) on the absorbance were used. The marks in Table 4 revealed that 1.0 mL of sodium hydroxide showed the highest absorbance value, therefore it was adopted for the subsequent experiments.

Table 4. **Effect of different amounts of various alkaline solutions on absorbance.**

Base solution (1M)	Absorbance/mL of base added					pH-range
	0.5	1.0	1.5	2.0	3.0	
NaOH	0.2363	0.4645	0.1363	0.3428	0.3637	11.9-13.2
KOH	0.1312	0.1102	0.0246	0.0639	0.0863	6.81-12.8
NH_4OH	0.1126	0.0395	0.0102	0.0106	0.0374	5.87-9.61
Na_2CO_3	0.2745	0.1788	0.0305	0.0957	0.0974	6.99-10.4
$NaHCO_3$	0.1720	0.0537	0.0313	0.0492	0.0530	5.32-9.97

Sequence of Addition

The effect of various sequences of the reaction components on the absorption of the azo-dye formed was also checked. The experimental results showed that the order of (NDP + $NaNO_2$ + HCl + γ-resorsolic acid + NaOH) was chosen as the optimum because of the best value of absorbance (A=0.4647). This sequence had been adopted in the previous and next experiments.

Time Effect on the Color Development

Three different concentrations of 50, 100 and 150 µg of NDP were used to study the effect of time on the color development of the resulting azo-dye at a wavelength of 436 nm. The absorbance was measured at varying times up to 90 min. The data in Fig. 4 show that the yellow azo-dye was developed after 5 min and there was no noticeable change appeared in absorbance for at least 90 min.

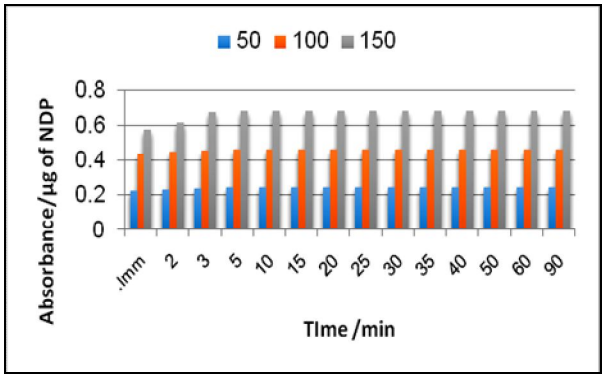


Figure 4. **Influence of time on color development of azo dye**

Influence of an Excipient Amounts

The selectivity of the suggested method was examined by measuring the absorbance of the solutions containing 100 µg of NDP and different quantities of 100, 250 and 500 µg of excipients such as glucose, lactose, Arabic gum, sorbitol and starch which often accompany pharmaceutical preparations. The results indicate that the ingredients did not interfere seriously and there was no significant difference.

Absorption Spectra

On treatment, a solution containing micro amounts of the diazotized NDP with 1.5 mL γ-resorsolic acid reagent in a basic solution of NaOH a yellow color product of azo dye was produced. The resulting dye displayed a maximum absorption peak at the wavelength of 436 nm against the blank solution (Fig. 5). The intensity of the yellow colour was directly proportional to the amount of NDP that originally existed in the sample solution.

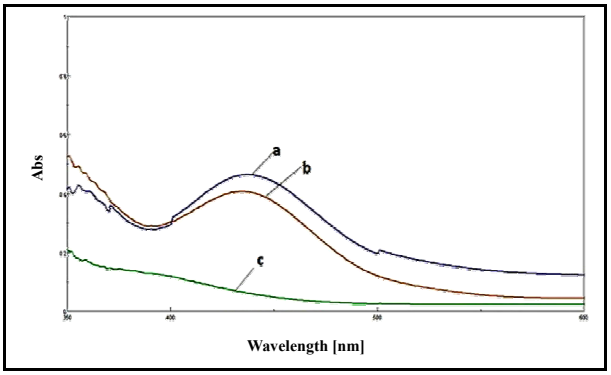


Figure 5. **Absorption spectra of 10 µg/mL of NDP : (a) against dw, (b) against blank solution and (c) blank against dw**

Linearity and Sensitivity

A linear calibration graph was constructed between the absorbance at 436 nm and the linearity was found to be in the concentration range of 1.0-40.0 µg/mL of NDP under the established experimental conditions (Fig. 6) with a value of determination coefficient (R^2) equal to 0.9988. The values of molar absorptivity, Sandell's index, LOD, LOQ, precision (RSD) and accuracy were calculated and the data are summarized in Table 5, which reveals that the developed method for the estimation of NDP is precise and accurate.

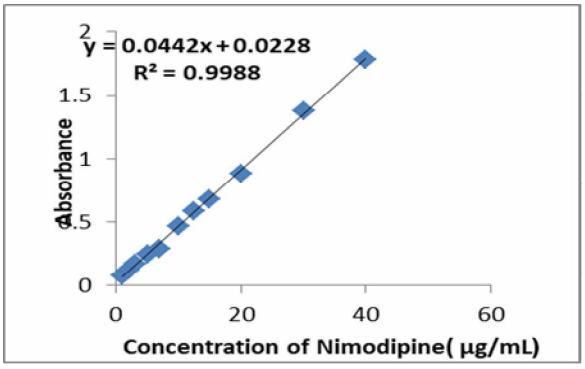


Figure 6. **Standard calibration curve for NDP estimation**

Table 5. **Optical characteristics and analytical data of the development method.**

Parameters	Value
Beer's law range (µg/mL)	1.0- 40
Molar absorptivity (L/mol.cm)	$1.8495x10^4$
LOD (µg/mL)*	0.0059
LOQ (µg/mL)*	0.0195
Relative error percent range (%)	-3.78% to2.22%
Recovery percent range (%)	97.95% to 99.08% better
RSD (%) (N=5)	better than 0.323%
Determination coefficient (R^2)	0.9988
Slop (a)#	0.0442
Intercept (b)#	0.0228
Sandell's index(µg/cm^2)	0.0226

***[31], # Regression equation (X = b + ac), where c is [NDP] in µg/mL.**

Nature of the Resulting Azo Dye

The molar ratio of the azo-dye formed between the diazotized NDP and γ-resorsolic acid was accomplished using the mole ratio and continuous variations methods (Job's method) [32]. In the continuous variation method, volumes of 0.5 to 2.5 mL of $2.3898x10^{-4}$ M of γ-resorsolic acid were coupled according to the recommended procedure with the corresponding complementary volume of $2.3898x10^{-4}$ M NDP solution to give a total volume of 3.0 mL and diluted to 10 mL with dw. Whereas, in mole ratio method, increasing amounts of 0.25 - 3.0 mL of $2.3898x10^{-4}$ M γ-resorsolic acid solution was added to a 1.0 mL of $2.3898x10^{-4}$ M of NDP and the absorbance was recorded at 436 nm after dilution to the mark with dw. The results in Fig.7 illustrate that the combining ratio of NDP to the reagent γ-resorsolic acid is 1:1. The evident stability constant (Ks) [33] of the resulting azo dye was also calculated and found to be $6.7892x10^6$ L/mol. This indicates that the azo-dye product has good stability.

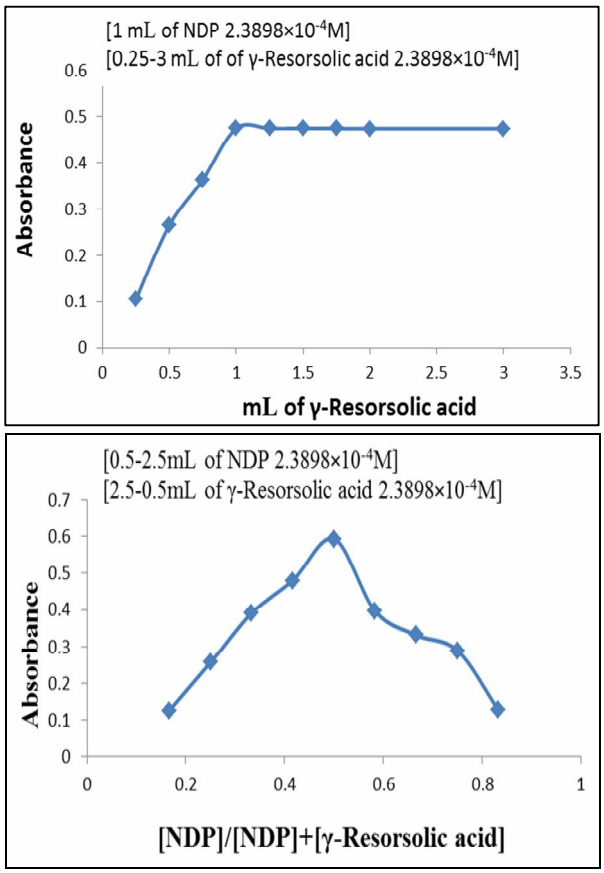
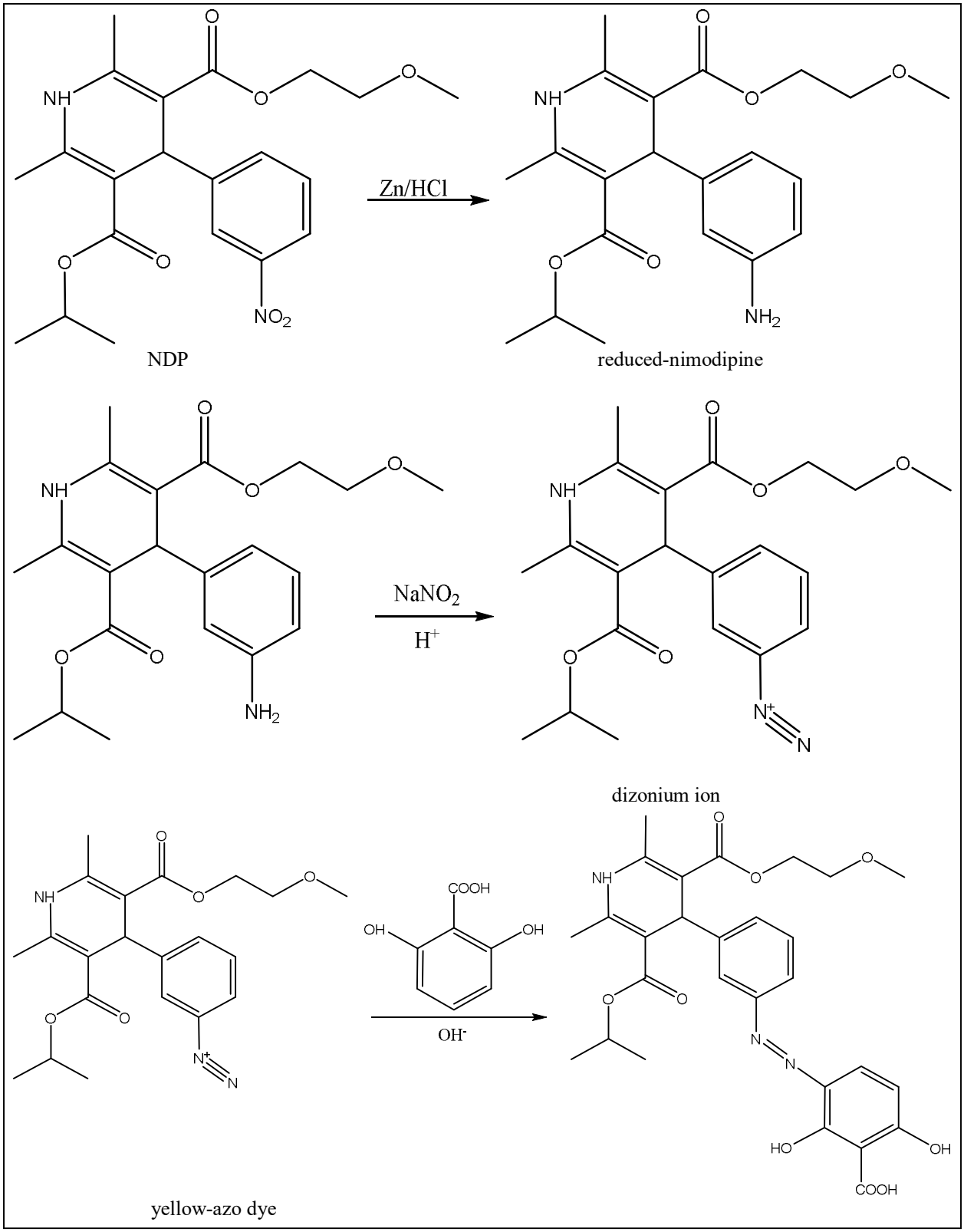


Figure 7. **Plots of (a)the continuous variation and (b)mole ratio methods for azo dye.**

According to the results of the stoichiometry study, the suggested chemical structure of the azo dye formed through the diazotization and coupling reaction of NDP with γ-resorsolic acid can be clarified in Scheme 2.

Mechanism of the Reaction

This reaction involved two steps that produced yellow azo dye. In the first step, the reduced form of NDP was treated with an equimolar nitrite solution in an acidic medium (HCl) and then underwent diazotization to form a diazonium ion. Second step, the diazotized NDP was reacted with a coupling agent of γ-resorsolic acid to give the colored azo-dye in an alkaline medium and the overall chemical reactions are clarified in Scheme 2.



Scheme 2. **Proposed reaction mechanism of NDP**

Applications

The applicability of the developed method for the estimation of NDP has been tested on commercially available pharmaceutical formulations (tablet) and biological fluids (urine and serum) at three different quantities 50, 100 and 150 µg NDP. The grades listed in Table 6 indicate that the developed method is suitable for assaying NDP with acceptable results.

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

Sample	NDP present (µg)	NDP Found (µg)*	R.E. (%)*	Recovery (%)*	RSD (%) (N=5)
Nimotap 30 mg/tablet (Germany)	50	48.94	-2.12	97.88	0.280
	150	148.7	-0.88	99.13	0.287
Urine	50	48.92	-2.16	97.84	0.323
	150	153.34	2.22	102.22	0.113
Serum	50	48.11	-3.78	96.22	0.324
	150	152.37	1.58	101.58	0.164

***Average of five estimations**

Evaluation of the Proposed Method

To evaluate the results of the developed method, the statistic factor t-test values has been investigated and the results in Table 7 reveal that the experimental t-value is less than the tabulated value at the 95% confidence level [34]. These results indicated that the difference is statistically not significant, which confirms the success of applying the developed method to assay 10 µg/mL of NDP in its pharmaceutical form (tablet) and biological fluids.

Table 7. **Estimation of NDP in the tablet and biological fluids.**

Sample	mL of sample	NDP Found (µg)*	R.E. (%)*	Recovery (%)*	RSD (%)*	t-exp.**
Nimotap 30mg/tablet (Germany)		97.81	-2.19	97.81	0.346	2.462
Urine	0.5	98.52	-1.48	98.52	0.5641	2.23
	1.0	98.28	-1.72	98.28	0.3081	2.14
Serum	0.5	97.27	-2.73	97.27	0.2404	1.72
	1.0	98.75	-1.25	98.75	0.4659	1.00

*Average of five estimations, **t-exp: (t-experimental), $\pm t = \frac{(\bar{X}-\mu)\sqrt{N}}{S}$, Tabulated t-value at 95% confidence level is equal to 2.776, degree of freedom (N=4)

Evaluation of the Results by Using Standard Addition Method

To prove the efficiency and credibility of the suggested method for assaying NDP and to ensure that it was free from the interference of additives, a standard addition method was used. The experimental results illustrated in Fig.8 and Table 8 indicate that there is a high agreement between the standard addition method and the proposed method for determining NDP in its pharmaceutical form (tablet).

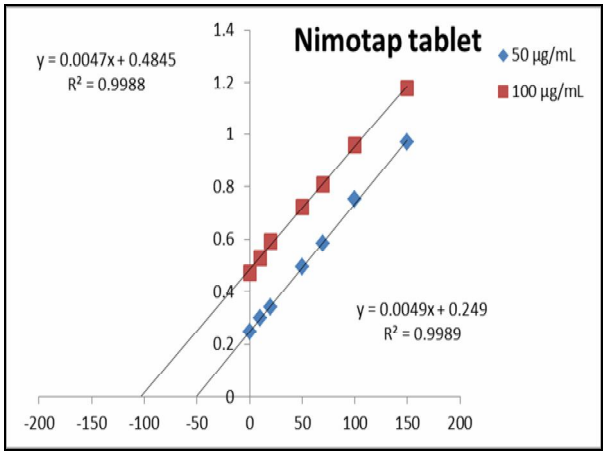


Figure 8. **Plot of standard addition method for the analysis of NDP in tablet**

Table 8. **Assay of NDP in commercial tablet using standard additions method.**

Drug	Certified value	NDP Found (µg)	NDP Present (µg)	Measured value	R.E. (%)	Recovery (%)
Nimotap (Germany)	30 mg/tablet	50	50.28	100.56	0.56	30.17
		100	99.12	99.12	-0.88	29.74

Comparison with other Spectrophotometric Methods

The present research compared with other reported methods in the literature using the same process (forming colored azo-dye), the results are summarized in Table 9.

Table 9. **Comparison of results with the reported method.**

Reagents	λ_{max} (nm)	Linear range (µg/mL) and εmax (L/mol.cm)	Sandell's sensitivity (µg/cm^2)	References
Phloroglucinol	410	0-25 $1.23x10^4$	0.034	[18]
Acetylacetone	420	0-35 $0.421x10^4$	0.0993	[19]
Citrazincic acid	440	0-35 $0.762x10^4$	0.0549	
β-Naphthol	555	0-10 $7.74x10^2$	0.0135	[20]
Recorcinol	480	2.0-35 $0.67x10^4$	0.0622	[21]
γ-Resorsolic acid	436	1-40 $1.8495x10^4$	0.0226	Proposed method

Conclusion

This search describes a simple and accurate spectrophotometric method based on the reaction of NDP through diazotization and coupling reaction using an available reagent of γ-resorsolic acid. The proposed approach has the advantages of being sensitive, low-cost, accurate and precise enough to replace the current spectrophotometric methods. It does not involve pre-extraction nor temperature control. The suggested approach is also selective and free from the common excipients; hence it can be used routinely for the estimation of NDP in tablets and biological fluids (urine and blood) with accepted recoveries.

Conflict of Interest

Trere is no conflict of interest .

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