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The Use of Charge Transfer Reaction for the Spectrophotometric Estimation of Nimodipine in the bulk, Pharmaceutical Formulation and Biological Fluids

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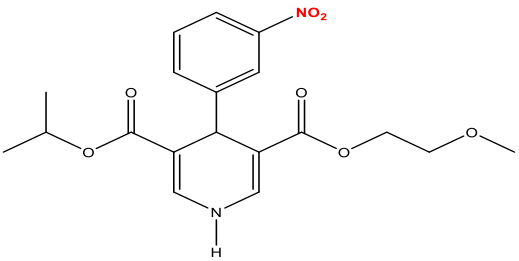
ABSTRACT

The present study deals with a sensitive, accurate and simple spectrophotometric approach for the valuation of nimodipine in the bulk , tablet and biological fluids. The procedure involved the reaction of the reduced nimodipine with 2,3-dichloro-5,6-dicyanobenzoquinone in borate buffer solution of pH9 to form a colored yellow-orange charge transfer complex. The resulting complex is soluble in water and showed a maximum absorption peak at 464 nm. All the variables which affected the conditions such as, influence of organic solvents, pH, buffer solutions, reagent concentration , reaction time and Beer′s law limits were studied carefully and adjusted. The optimal conditions showed that the color of charge transfer complex is stable for at least 60 minutes. The standard calibration curvature was linear in the variety from 1.0-40 µg/ml with a good value of determination coefficient (R^2= 0.9989). The molar absorptivity was calculated and set up to be $1.7031x10^4$ l/mol.cm. The detection limits and quantitation limits were also estimated and found to be 0.00512 and 0.01737 µg/ml, correspondingly. The approach was established by estimating of nimodipine in the pharmaceutical tablet and biological fluids. The precision (RSD) was calculated to be better than 1.556%, whereas the values of recovery percent and relative errors were in the range of 96.33% to 101.6% and **-**3.67% to-1.58% respectively, without interfering from any common excipients present the samples. The nature of the resulting complex has been studied between the reduced nimodipine and 2,3-dichloro-5,6-dicyano-benzoquinone reagent and was equal to 1:1.

***Keywords*:** Nimodipine, 2,3-Dichloro-5,6-dicyanobenzoquinone, Charge transfer complex, Spectrophotometry.

INTRODUCTION

Nimodipine (NDP) is a 1,4-dihydropyridine calcium channel blocker that acts by relaxing the arterial smooth muscle (Zaijin *et al.*, 2019). NDP is famous for its favored action on cerebral vessels than other agents within the same class (Sherif *et al.*, 2020). It's likely cytoprotictive effects by reducing calcium inflow into nerve cells (Rang *et al.*, 2007). The IUPAC name of NDP is 3,5-pyridinedicarboxylic acid,1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-,2-methox-yethyl 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) (British Pharmacopeia, 2022).



Scheme 1. Chemical structure of NDP.

A number of analytical techniques have been described in the literature for the determination of NDP in the pharmaceutical forms and biological fluids. These techniques included, RP-HPLC (Swetha *et al.*, 2021), LC-electrospray tandem mass spectrometry (Nirogi *et al.*, 2006), LC-tandem mass spectrometry (Fadumo *et al.,* 2020) , LC-MS/MS (Sonani *et al.,* 2020), spectrofluorometry (F.Belal *et al.*, 2003), HPLC-UV (Camilla *et al*., 2022) and (Atefeh *et al*., 2017), HPLC-ESI-MS (Ying *et al.*, 2010), differential pulse voltammetry using modified reduced graphene oxide composite (Ting and Geng, 2023), square wave voltammetry (SWV) (Al-Montaser *et al.*, 2022), cathodic adsorptive stripping voltammetry (Vinod *et al*., 2011), UV-spectrophotometry (Rajesh *et al.*, 2018) and atomic absorption spectroscopy (Mevlude and Sezen, 2005). Most of these methods were required critical working conditions, heating step and an expensive equipment and a skilled operation.

Visible spectrophotometric methods still utilized widly due to its sensitivity, selectivity, rapidity and economic. Therefore, different UV-visible spectrophotometric methods were used for estimating NDP in aqueous medium. Most of these approaches involved diazotization of the reduced nimodipine and coupling with versus reagents , such as, phloroglucinol (Hemavathi and Hosakere, 2013), acetylacetone, diphenylamine, citrazinic acid and chromotropic acid (Hosakere *et al*., 2011), resorcinol (Revansiddappa *et al.*, 2011). Others based on the ion-pair extraction-spectrophotometry (Trung *et al.*, 2022), condensation reaction with p-anisaldehyde (Mostafa *et al.*, 2015), coupling reaction of NDP with vanillin reagent in acidic medium (Mohamed *et al.*, 2013), oxidation-reduction reaction with potassium permanganate (Askal *et al.*, 2010), bleaching reaction using indigo carmine dye and N-bromosuccinimide as oxidant (Mohamed *et al.*, 2013). 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, applicable to higher concentrations of NDP and high detection limit value or required solvent extraction. The focal aim of this approach is to create an economic, rapid, simple and precise spectrophotometric procedure via charge transfer (C-T) complex formation using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) for assaying NDP in the bulk, pharmaceutical formulation and biological fluids.

EXPERIMENTAL

Instrumentation

All spectral measurements were carried out using a Jasco V630 UV-Vis double beam spect-rophotometer (Japan) with 1.0-cm matched fused silica cells, and the pH measurements of the solutions were made using a professional device Bp3001 bench top PH meter.

Preparation of solutions

All chemicals and reagents were of analytical grade.

Drug sample

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

Standard reduced NDP solution (100 µg/ml) (2.3898×10^{-4}M)

A 10 mg of pure NDP was accurately weighed, transferred into 100 ml beaker and dissolved in 5 ml of methanol. A 5 ml of 4N hydrochloric acid and 1g of zing powder were then added, and the solution was shaken for about 20 minutes thoroughly, and the volume was adjusted up to the mark with distill water. Then, the final solution was filtrated using Whatman No.41 filter paper (Hoskare *et al.,* 2011).

DDQ solution (3×10^{-3}M)

This acceptor solution was prepared daily by dissolving 0.0277g of DDQ in absolute ethanol, and the volume was diluted with the same solvent in a 100-ml calibrated flask.

Sodium tetraborate solution (0.025)M

A 0.9534 g of sodium tetraborate was dissolved in distilled water, and diluted to the mark in a 100 ml calibrated flask.

Boric acid solution (5%)

This solution was prepared by weighing 5 g of boric acid in a little amount of hot distill water, and heated for 10 minutes after cooling, the solution was diluted to 100 ml in calibrated flask using the same solvent.

Sodium hydroxide solution (0.1M)

One ampoule of 1M standard sodium hydroxide (BHD) was diluted to one liter with distilled water and stored in a plastic bottle.

Borate buffer solution of pH9

It was obtained by mixing 50 ml of (0.025M) sodium tetraborate with 0.8 ml of (0.1M) sodium hydroxide in a 100-ml calibrated flask and diluted to the mark with distill water (Perrin,1974). Various borate buffer solution ranging from pH 8.2 - 9.6 was prepared by using different quantities of sodium tetraborate (0.025 M), 0.1M NaOH and 5% of boric acid all these values of pH were adjusted by using the pH meter.

Essential procedure

An aliquot of reduced standard NDP solution containing 1.0-40 µg/ml of NDP and 2 ml of $1x10^{-3}$M DDQ solution was transferred into a sequence of 10 ml calibrated flasks and mixed well. To each flask 2 ml of NaOH (0.1M) and 1.5 ml of borate buffer (pH9) solution were added. The contents of the flasks were shaken, left for 10 minutes and finally filled to the mark with distilled water. The absorbance of each sample of the resulting (C-T) complex was measured at 464 nm at room temperature against corresponding reagent blank which containing all materials except NDP.

NDP tablet solution (100 µg/ml)

Ten tablet of nimodipine each one contains 30 mg of NDP were weighed exactly and ground into a fine powder . From this, powder an equivalent to 10 mg of NDP was accurately weighed and dissolved with 5 ml of methanol followed by adding 5 ml of 4N HCl and 1g of zinc dust. The contents were shaken continuously for about 20 minutes, and the volume was completed with distill water to 100 ml in a calibrated flask. After that the solution was then filtered using Whatman No.41 filter paper. An aliquot of the filtrate solution was analysed by following the proposed procedure to estimate the drug amount.

Preparation of spiked biological fluids

Serum and urine samples were provided from several healthy volunteers .To 1 ml of serum, 5ml of absolute acetonitrile was added to deproteinized it , the sample solution was then introduced in a centrifuge for 5 minutes at 2500 rpm. The supernatant was used for investigating recovery. Spiked urine was 50-fold diluted with distill water and a convenient amounts of NDP standard solution were added to 0.5 and 1 ml of the treated serum and urine solutions. The analysis of NDP was followed as in the recommended procedure (Akram and Ismail, 2012) and (Akram and Wafaa,2012).

RESULTS AND DISCUSSION

Chemistry of the colored complex

This work involve charge-transfer C-T complex formation between NDP as n-donor and DDQ as (π)-acceptor in non-polar solvent. DDQ is a strong acceptor due to the electron withdrawing effect of the two cyano and two chloro atoms. The resulting coloured product of NDP-DDQ complex exhibited a maximum absorption at 464 nm this may attributed to the formation of DDQ radical anion resulting from complete transfer of n-electron from NDP to the DDQ acceptor.

Optimization conditions

All experimental variables affecting mainly on the sensitivity and stability of the C-T complex formation were optimized. The subsequent experiments were performed using 100 µg of standard reduced NDP solution in a final volume of 25 ml calibrated flask and the absorbance of the resulting C-T complex was recorded at the wavelength of 464 nm against its blank solution.

Choosing the suitable solvent

In order to provide maximum sensitivity of the method, the effect of various organic solvents such as, methanol, ethanol, propanol, acetonitrile and as well as water on the solubility of NDP , DDQ and C-T complex was checked. The investigation was based on the measuring the absorbance of the sample solution at 464 nm against its blank solution and the results are summarized in Table (1).

Table (1): Organic solvents effect on the solubility of NDP, DDQ and on absorbance of C-T complex

Type of solvent			Absorbance	λ max (nm)
NDP (100 µg /ml)	DDQ ($1x10^{-3}$M)	Final dilution		
Methanol	Methanol	Methanol	Turbid	-------
Ethanol	Ethanol	Ethanol	0.0454	429
Propanol	Propanol	Propanol	0.1085	423
Methanol	Methanol	Water	Turbid	------
Ethanol	Ethanol	Water	0.0704	365
Propanol	Propanol	Water	0.0724	385
Water	Methanol	Methanol	-0.0195	410
Water	Ethanol	Ethanol	-0.0333	438
Water	Propanol	Propanol	0.0798	417
Water	Methanol	Water	Turbid	------
Methanol	Ethanol	Water	**0.2711**	**464**
Water	Propanol	Water	0.1402	419
Water	Water	Water	0.0205	365
Acetonitrile	Acetonitrile	Acetonitrile	Turbid	------
Acetonitrile	Acetonitrile	Water	Turbid	------
Water	Acetonitrile	Acetonitrile	0.0512	412
Water	Acetonitrile	Water	0.1417	423

The results in Table (1) reveal that the color intensity is increased when using methanol and ethanol as solvents for NDP and DDQ. It is noticeable in this context too, that the final dilution of the mixture reaction to the mark with water gives good results. But, on using other solvents led to decrease the values of absorbance. Therefore, the system of using methanol and ethanol has been recommended to this work.

Effect of pH and the type of buffer solution

It was found that the effective formation of the product depends on the pH value and the type of buffer solution. The influence of different pH values 5-12 on absorbance was carried out. The results in Table (2) show that the C-T complex is consistence when the final pH of solution is equal 9.

Table(2) : Influence of pH on absorbance

pH	**5.0**	**6.0**	**7.0**	**8.0**	**9.0**	**10.0**	**11.0**	**12.0**
Absorbance	0.0521	0.0271	0.0308	0.2157	**0.2721**	0.1421	0.1152	0.0972
Λmax (nm)	385	390	379.0	418	**464**	423	424	421

The influence of various buffer solutions of pH9 such as, ammonia, carbonate, phosphate and borate (Perrin, 1974) were also investigated. According to the results listed in Table (2), a good color contrast and the highest absorbance were obtained with borate buffer solution (sodium tetraborate).

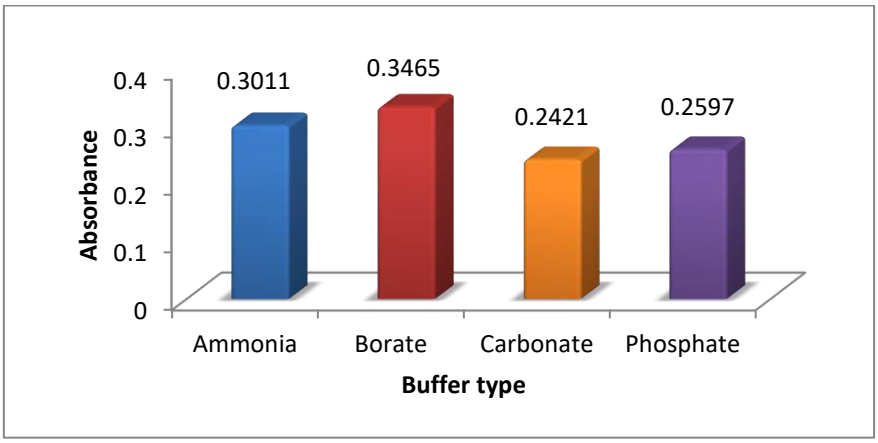


Fig.(2) : Influence of various buffer solutions of pH 9 on absorbance

The influence of different pH values 8.6 - 9.8 of sodium tetraborate buffer solution has been also examined. The results in Table (3) clarify that the highest absorbance of the solutions was obtained at pH value from 9.0 to 9.4.

Table(3) : Influence of different pH values of borate buffer solution on absorbance

pH range	8.6	8.8	**9.0**	**9.2**	**9.4**	9.6	9.8
Absorbance at 464 nm	0.2804	0.2903	**0.3469**	**0.3465**	**0.3460**	0.3458	0.2628

Influence of the amount of borate buffer solution (pH9)

The influence of different portions 0.5-2.0 ml of borate buffer solution on the absorbance of C-T complex solution was carried out. The results are illustrated in Fig. (3) and indicated that the volumes of 1.5 and 1.75 ml of borate buffer solution are the optimal due to their highest sensitivity, therefore, these volumes have been selected for the subsequent experiments.

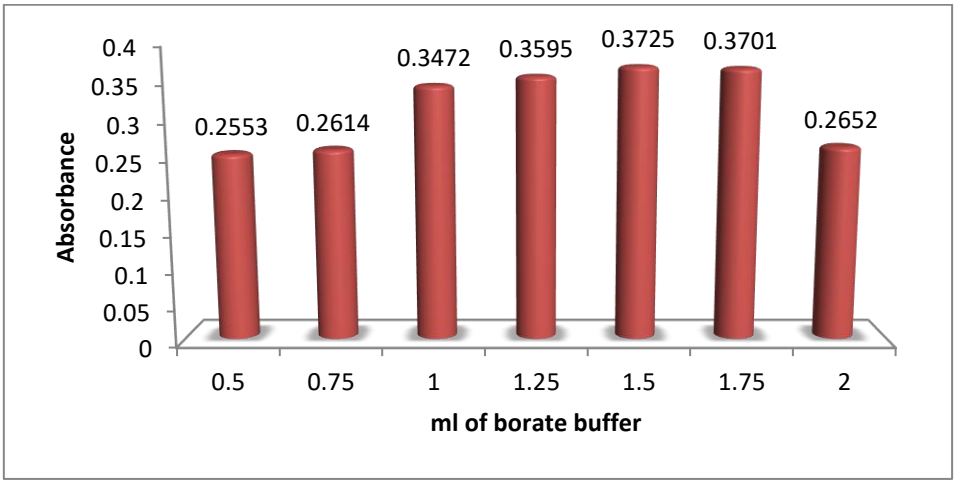


Fig. (3) : Influence of borate buffer amount on absorbance

Effect of DDQ concentration

The influence of varying amounts 0.5-2.5 ml of DDQ ($1x10^{-3}$M) on absorbance of solutions containing different quantities 25-50 µg of reduced NDP was investigated. The data are summarized in Table (4) and revealed that the highest absorbance with a good correlation coefficient (R^2= 0.9997) was achieved by utilizing 2 ml of DDQ ($1x10^{-3}$M) solution, so it was relied upon for the subsequent experiments.

Table(4) : Effect of DDQ concentration on absorbance

mL of $1x10^{-3}$M DDQ	Absorbance / µg of NDP				R^2
	25	**50**	**100**	**150**	
0.5	0.0446	0.1356	0.2571	0.3954	0.9962
1.0	0.0629	0.1689	0.3720	0.5268	0.9916
1.5	0.0688	0.1785	0.3915	0.5487	0.9942
2.0	0.0825	0.1958	**0.3972**	0.6108	**0.9997**
2.25	0.0716	0.1723	0.3918	0.5727	0.9982
2.5	0.0591	0.1588	0.3256	0.4863	0.9986

Temperature and reaction time effect

The effect of temperature on the absorbance of the resulting C-T complex was carried out at three different temperatures (room temperature, 40 and 50℃) using a thermostatic water bath and at different periods of times before dilution the flasks. According to the results in Fig.(4), the reaction of NDP with DDQ to form C-T complex does not temperature dependent because , the absorbance reached its maximum value at 10 minutes and remains nearly constant to 40 minutes at room temperature (25°C). Thus, 10 minutes was selected as the optimum reaction. But, at higher temperature of 40 and 50℃ the absorbance was decreased and the turbidity became clearly observed in the solutions and this may be attributed to the dissociation of the complex.

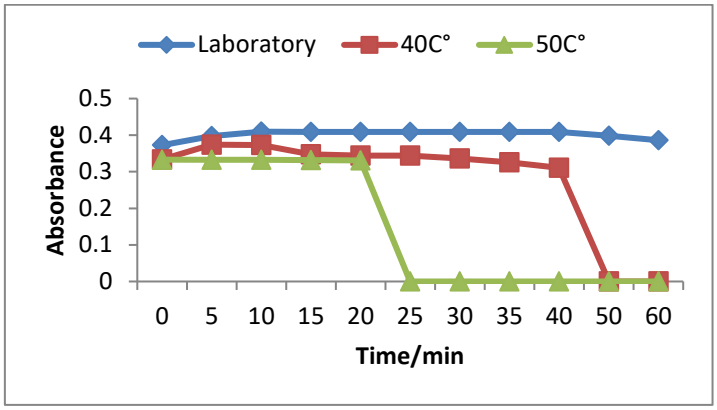


Fig. (4) : Influence of temperature and time on absorbance of C-T complex

Effect of order of addition

The effect of order addition of the reactants on the absorbance of the resulting C-T complex was also diagnosed. The experimental results indicated that the most favorable order of addition was (NDP + DDQ + NaOH + Borate buffer) due to it's the highest color intensity and development of maximum absorbance (A=0.4095), while other sequences lead to decrease the absorbance values**.**

Time effect on the colour development

The effect of time on the absorbance of the C-T complex was studied at different time periods. The resulting yellow-orange colour of the complex after dilution the solution remains stable for at least 60 minutes. The results are illustrated in Fig.(5).

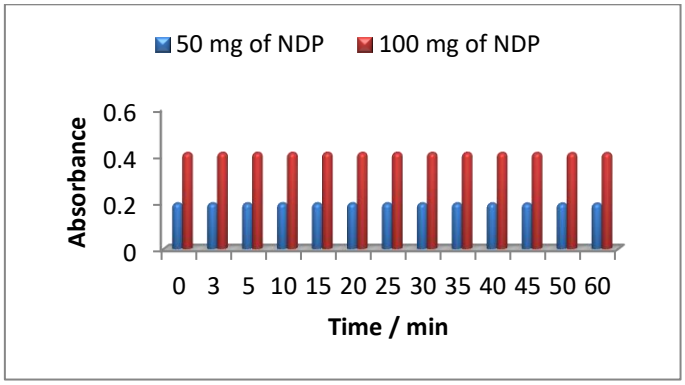


Fig. (5) : Time effect on the colour development

Summary of optimum conditions and final absorption spectra

According to the optimal reaction conditions which diagnosed for the estimation of NDP and confirmed in Table (5), a microamount of NDP was treated with DDQ reagent in an alkaline solution at pH9 using borate buffer solution to form a yellow soluble C-T complex. The resulting complex exhibits a maximum absorption peak at 464 nm against the blank solution. The final absorption spectra of NDP estimation via charge-transfer reaction is illustrated in Fig.3.

Table (5) : Summary of the optimal conditions

Parameters	Optimum conditions
λmax (nm)	464
ml and concentration of DDQ	2 ml , $1x10^{-3}$M
ml of borate buffer solution (pH9)	1.5
ml of 0.1M NaOH	2
Time of reaction (min.)	10
Temperature (℃)	Laboratory temperature
Stability period (min.)	60
Reaction medium	Water

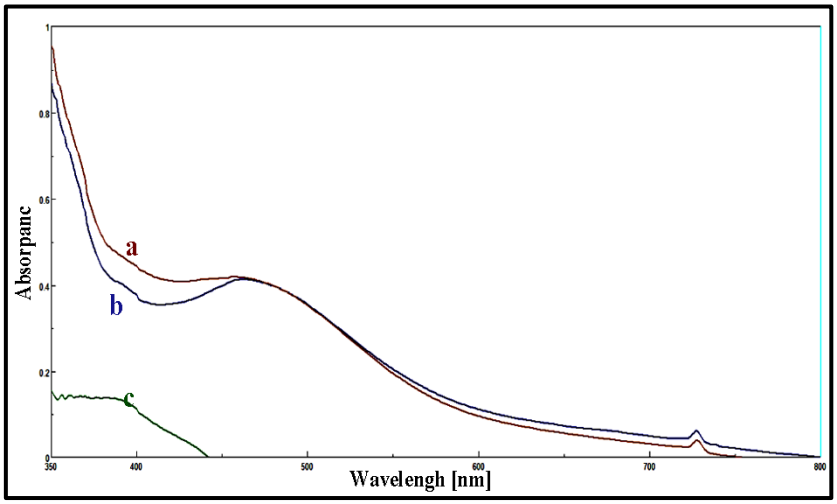


Fig. (6): Final absorption spectra of 10 µg/ml of NDP recorded (a) against distilled water, (b) against blank solution, (c) blank against distilled water.

Standard Calibration plot

A linear standard calibration plot was obtained and obeyed to Beer's law in the concentration range of 1.0 - 40 µg/ml NDP with an excellent determination coefficient 0.9989 (Fig. 7). The index of Sandell's sensitivity and molar absorptivity were evaluated and found to be 0.02456 µg/cm^2 and 1.7031×10^4 l/mol.cm, respectively which indicate that the method is sensitive. The limit of detection and limit of quantitation values were also evaluated and found to be 0.00512 and 0.01737 µg/ml , correspondingly (Valcarcel Cases *et al.,* 2018**).**

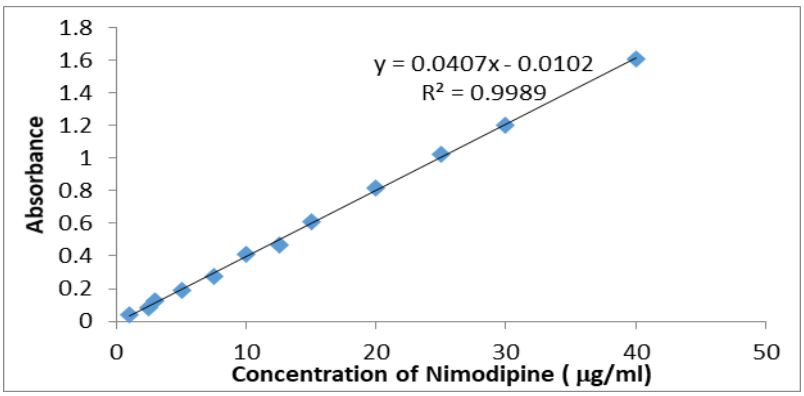


Fig.(7):Standard calibration plot for determining NDP using recommended method

Stoichiometry of C-T complex

Continuous variation and slop ratio methods (Delevie, 1997) have been applied to establish the composition of the C-T complex which created from the reaction of NDP with DDQ. In both methods an equal concentrations ($2.3898x10^{-4}$ M) of the drug and the reagent DDQ were used.

In continuous variation method, an increasing volumes 0.4 -3.6 ml of the reduced NDP solution were reacted according to the suggested procedure with the corresponding complementary volumes of DDQ solution to give a total volume of 4 ml and diluted to 10 ml with distilled water.

In slop ratio method two standard calibration curves were plotted. The first curve was prepared by adding different volumes 0.5-4 ml of DDQ solution to a fixed volume (1ml) of NDP solution, and in the second curve, an increased volumes 0.5-4 ml of NDP solution were added to a 1 ml of DDQ solution using 10 ml calibrated flasks. the absorbance was recorded at 464 nm after dilution to the mark with distilled water. The results of both methods are illustrated in Figs.8&9, indicated that the resulting C-T complex was formed by a 1:1 combining ratio of NDP to DDQ.

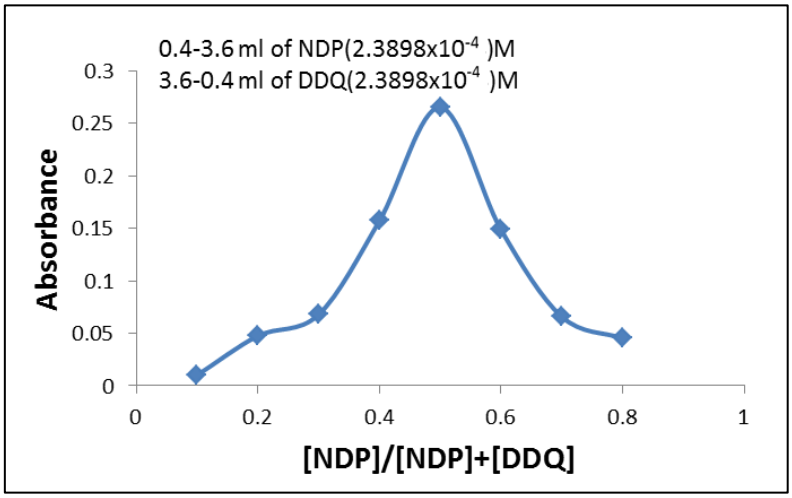


Fig. (8) :Plot of the continuous variation method

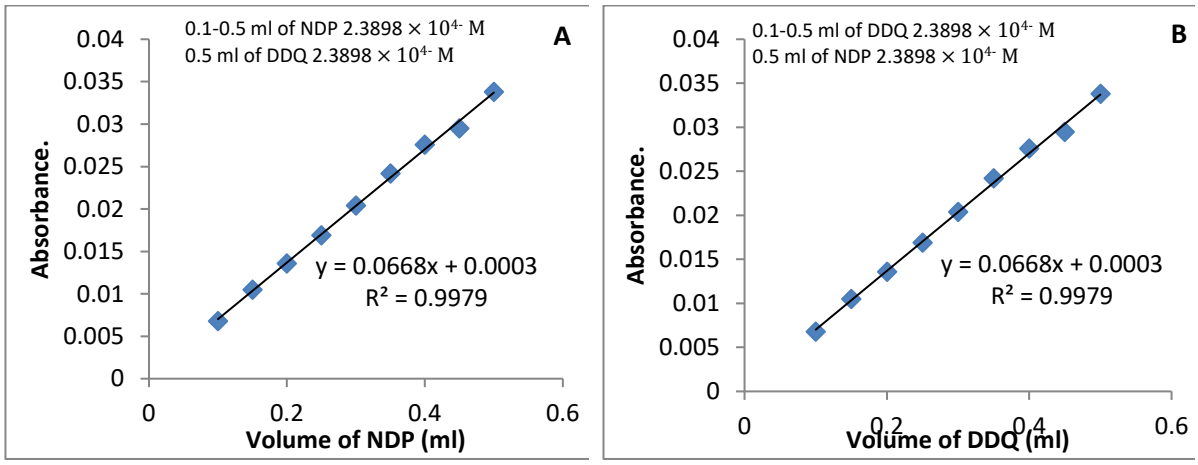


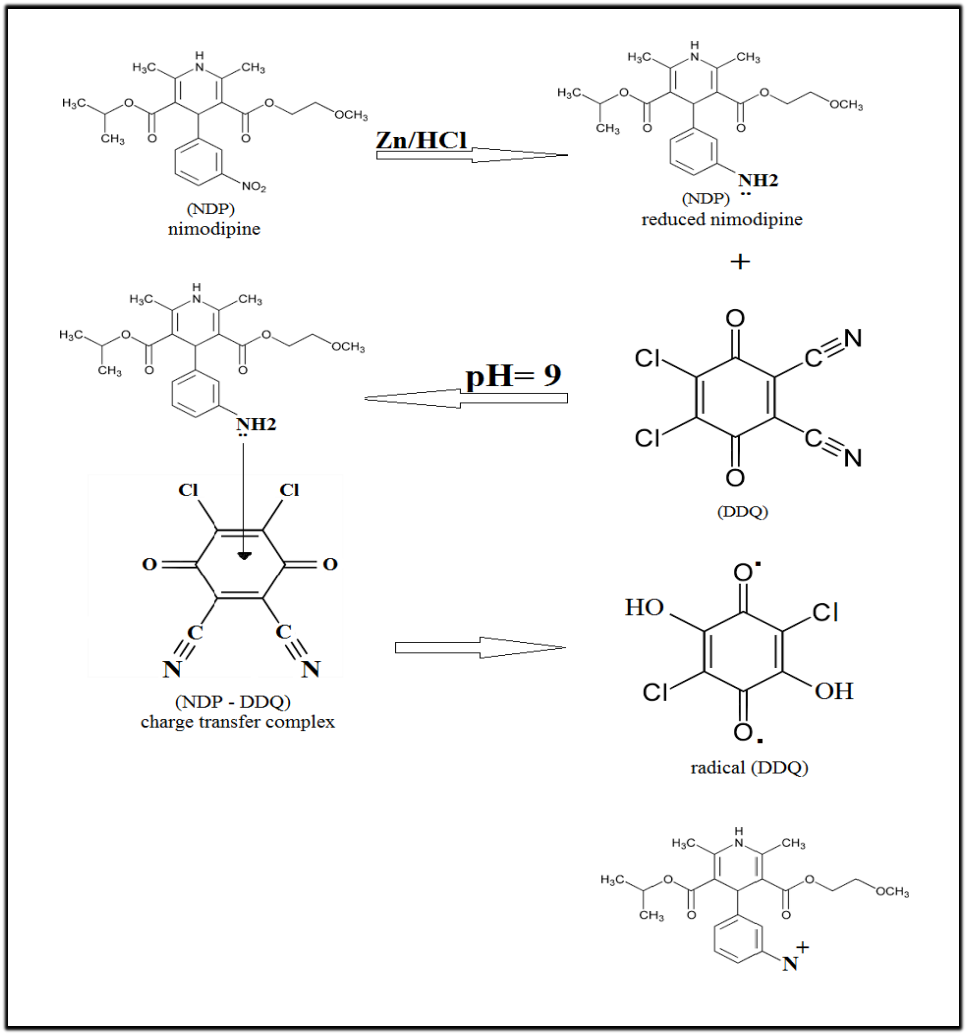
Fig. (9): Plots of slope ratio method

Equations of the C-T complex formation reaction

This method involves charge-transfer (C-T) complex formation between NDP as n-donor and DDQ as π -acceptor in non-polar solvent . An intense yellow-orange colored product was formed due to the interaction between NDP-DDQ in methanol-ethanol-water solvent system .

First step: Includes reduction of nitro group in nimodipine to amino group using zinc dust in acidic solution of hydrochloric acid(4N).

Second step: Includes the interaction of NDP with DDQ in presences of borate buffer of pH 9 to produce a yellow–orange C-T complex according to (Hemavathi and Hosakere, 2013) and (Gamal, *et al.,* 2022). The chemical structure of resulting C-T complex can be suggested as the following chemical reactions in Scheme (2).



Scheme 2: The chemical reactions of NDP-DDQ complex formation

Application of NDP estimation by applying the suggested method

In commercial tablet

The proposed work was applied for the estimation of NDP in nimotap (30 mg) tablet at three different quantities 25, 100 and 200 µg of NDP. The consequences are summarized in Table (6), show a satisfactorily accurate and precise as indicated by the excellent recovery% and RSD%.

In biological fluids

This work was attempted for estimating NDP in serum and urine samples. In this application known amounts of NDP (25,100 and 200 µg) were added to the sample solutions and the results are listed in Table (6) and proved that the method can be used for determining NDP in biological fluids with an acceptable results.

Table (6) : Assay of NDP in tablet and biological fluids

Sample	NDP found* (µg)	Recovery* (%)	R.E*. (%)	RSD (%) (N=5)
Nimotap 30 mg/tablet (Germany)	24.65	98.60	-1.40	0.929
	99.12	99.12	-0.88	1.297
	197.51	98.75	-1.245	1.079
Urine#	24.60	98.40	-1.60	0.936
	97.20	97.20	-2.80	1.044
	203.15	101.6	1.58	1.060
Serum#	24.30	97.20	-2.80	1.003
	96.33	96.33	-3.67	1.556
	202.86	101.43	1.43	1.001

*Average of five estimations, # 1ml of sample solution was used

Evaluation of the development method for estimating NDP in tablet and biological fluids

To evaluate the results of the suggested method for estimating NDP in tablet and biological fluids, a t-test and F-test were relied upon by comparing it with a method published in the literature (Hemavathi and Hosakere, 2013). The results in Table (7) confirmed that the t and F experimental values are less than the t and F-tabulated values at the 95% confidence level (Christian *et al.,* 2014).

Table (7) : Evaluation of 100 µg NDP estimation in the tablet and biological fluids

Sample	NDP Found (µg)*		Recovery* % ± RSD%		t- value[a]	F-value[a]
	Present method	Literature method	Present method	Literature method		
Nimotap 30 mg/tablet (Germany)	98.73	97.49	98.73 ± 1.14	97.49 ±1.31	0.229	0.86
Urine	97.20	96.86	97.20 ± 1.17	96.86 ±1.44	1.62	1.79
Serum	98.33	96.57	98.33 ± 1.46	96.57 ±1.61	1.30	0.63

*Average of five estimations,

Tabulated t-value and F-value at 95% confidence level are equal to 2.306 , and 6.39 respectively,

Degree of freedom (N=8) for t-value and (N=4) for F-value.

The results in Table (7) reveal that there is no significant difference between the development and the published methods.

Validity of the proposed method

In order to check the selectivity and validity of the suggested method for analysis of NDP in tablet, a standard addition method was applied for this purpose. The results are illustrated in Fig.(10) 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.

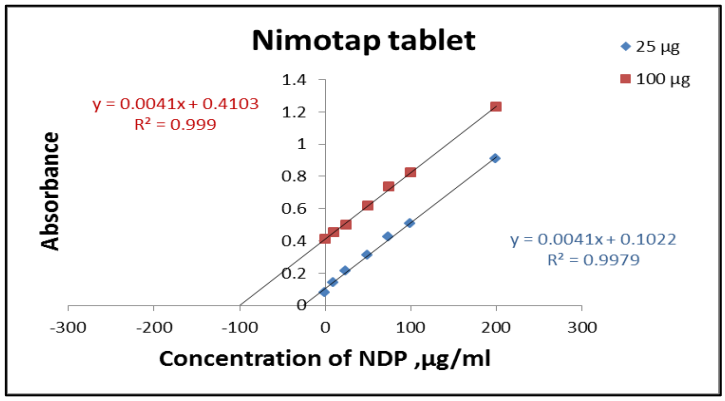


Fig. (10) : Standard addition method for analysis of NDP in tablet

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

Drug	Certified value	NDP		Recovery(%)	R.E. (%)	Measured value (mg)
		Present (µg)	Found(µg)			
Nimotap (Germany)	30 mg/tablet	50 100	24.93 100.07	99.72 100.07	-0.28 0.07	29.92 30.021

Conclusions

A DDQ reagent was used to develop a sensitive spectrophotometric method for the estimation of NDP in the bulk, pharmaceutical form and in the biological fluids through a charge transfer reaction. The method was found to be simple, economical, selective and does not required solvent extraction steps or temperature control. The method is being accurate and precise enough to be successfully applied for the analysis of NDP in commercial tablet and samples of urine and serum with accepted recoveries.

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