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Cloud-Point Extraction and Spectrophotometric Determination of Nifedipine in Pharmaceutical Dosage Forms

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ABSTRACT

A simple, sensitive and eco-friendly cloud point extraction (CPE) technique was suggested for spectrophotometric assay of nifedipine (NIF) in pure and pharmaceutical dosage forms. The red colored product results from oxidative coupling reaction between reduced NIF and 4-aminoantipyrine (4AAP) was extracted using non-ionic surfactant (TritonX-114) and then determined at maximum wavelength 554 nm using visible spectrophotometer. All the factors that affected the efficiency of extraction such as concentrations of reagents and surfactant, time of extraction and temperature were studied. Using resultant optimum conditions, the linearity range of calibration curve was 0.8-45 µg/mL (r = 0.9976). The detection and quantification limits were of 0.395 and 1.317 µg/mL respectively with relative standard deviation best than 2%. The method was applied for assay of NIF in pharmaceutical dosage forms with percentage recovery of 99.18% and preconcentration factor about 20. The molar ratio of the result product was studied by mole ratio method using equimolar concentration of reactants indicated that the complex formed between the reduced NIF and 4AAP was 1:1 respectively. Suggested CPE method was successfully applied for the estimated of NIF in pharmaceutical tablets. The results obtained are compared with UV method (standard method) and the results indicated insignificant difference between two methods.

Keywords: **Nifedipine, Cloud-point extraction, spectrophotometric method, TritonX-114. Pharmaceutical dosage forms**

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INTRODUCTION

Nifedipine (NIF) is chemically designated as 3, 5-dimethyl2, 6-dimethyl-4-(2-nitrophenyl)-1, 4-dihydropyridine-3, 5- dicarboxylate [1]. It is mainly used as channel blocker for calcium and in the managing of angina pectoris, hypertension, and some other cardiovascular disorders [2]. Several analytical methods have been reported for assay of NIF in different samples including voltammetry [3], HPLC [4], gas chromatography [5], spectrofluorometry [6], flow injection spectrophotometry techniques [7-9]. Cloud point extraction (CPE) combined with spectrophotometric detection is an important technique for extraction and analysis different species as an alternative of using expensive equipments [10, 11]. CPE technique has several advantages in comparison with other extraction techniques, such as simplicity, high recovery, low consumption of organic solvents with good enrichment factor [12, 13]. No article was reported in literatures relating to the estimation of NIF drug utilizing CPE-spectrophotometric method as a result, the present work involved a new method for the extraction and estimation of NIF based on the oxidative coupling reaction of its reduced form with 4AAP after oxidation with $NaIO_4$ and extracted the product using CPE technique.

Experimental

Apparatus

A single beam spectrophotometer (Shimadzu/ UV-1800 Japan) was used for establishment all absorption spectra and absorbance values of analyte. The measurements of absorbance were performed by a quartz cell (Cecil, 50 µL internal volume and 1 cm path length) for cloud point extraction measurements, a silica cells (1 cm) had been used. A thermostatic water bath expert (England) was used of CPE experiments. A centrifuge (Hettich, EBA 21) with 50 mL calibrated centrifuge tubes were used for separation process.

Reagents and solutions

The chemical compound used in this work were highly purify and the distilled water used for all working and preparation steps. NIF (Pharmaceutical grade, Sigma chemical co., Germany) and pharmaceutical tablets were obtained from local drugstore (Adalat, tablet containing 30 mg of NIF, Payer, Pharma AG, Germany).

Solution of reduced NIF (500 µg/mL)

A reduced stock solution of NIF was prepared according to previous suggested procedure [14]. A 0.0500 g of NIF was dissolved in 50 mL of ethanol and transferred into beaker of 125 mL. A reducing agent (20 mL of distilled water, 20 mL of HCl (37%w/w), and 3 g of zinc powder) were used to perform the reduction process, then the content was allowed to stand at room temperature (25°C, for 15 min), then filtered into 100 mL volumetric flask. The residues washed with distilled water and the solution was finally diluted to the mark with distilled water. Working solution was prepared by dilution with distilled water.

4-Aminoantipyrine ($5x10^{-3}$M)

This solution was daily prepared by dissolving 0.1016 g of 4AAP (Merck) in distilled water and diluting to 100 mL with the same solvent in volumetric flasks.

Sodium periodate solution (0.1 M)

The solution of oxidant was prepared by dissolving 2.139 g of $NaIO_4$ (Merck) with distilled water in 100 mL volumetric flask and diluting to mark with the same solvent.

Triton X-114(4% v/v)

For preparation a surfactant solution,4 mL of Triton X-114 (purity > 99.9%, Fluka) was dissolved in distilled water and diluted to mark in 100 mL volumetric flask with the same solvent.

General extraction and determination procedure

In 10 mL volumetric flasks, an increasing amount of NIF standard (0.8-45 µg/mL) or sample solution, 3 mL of 4AAP solution ($5x10^{-3}$M), 1 mL of NaOH (0.2 M), 1.5 mL of sodium periodate (0.1 M), and 3 mL of Triton X-114 (4% v/v) were added, mixed and dilute with distilled water. Into a 10 mL centrifuging tube, the content of the flasks was transferred and then kept in the thermostatic bath (at 65°C for 15 min). The separation of two phases was accomplished by centrifugation (3500 rpm for 5 min) and cooled down in an ice bath (to enhance the viscosity of the lower phase). The aqueous phase was removed by simple decantation. The remain phase (surfactant-rich phase) which comprises the red complex was dissolved with 0.8 mL of ethanol. The absorbance of the product was measured at 554 nm against a reagent blank.

Samples preparation of pharmaceutical for NIF detection

Twenty tablets of commercial NIF (adalat-30 mg) were weighted and grinded then solid powder equivalent to NIF (50 mg) dissolved in a volume of 30 mL ethanol. This solution mixed well, filtered into a volumetric flask (50 mL), washed and made up to the mark with ethanol. Then into a beaker of 150 mL, the previous solution transferred and reduced as described under general procedure. More diluted solutions of pharmaceutical samples were prepared by simple dilution with distilled water.

Results and discussion

Absorption spectra

The absorption spectrum of the reduced NIF-4AAP product in Triton X-114-rich phase expressions a maximum absorbance at 554 nm against reagent blank (Figure1), therefore, the wavelength of 554 nm for color product was chosen all over this study. This reaction was adopted to determine NIF using CPE method.

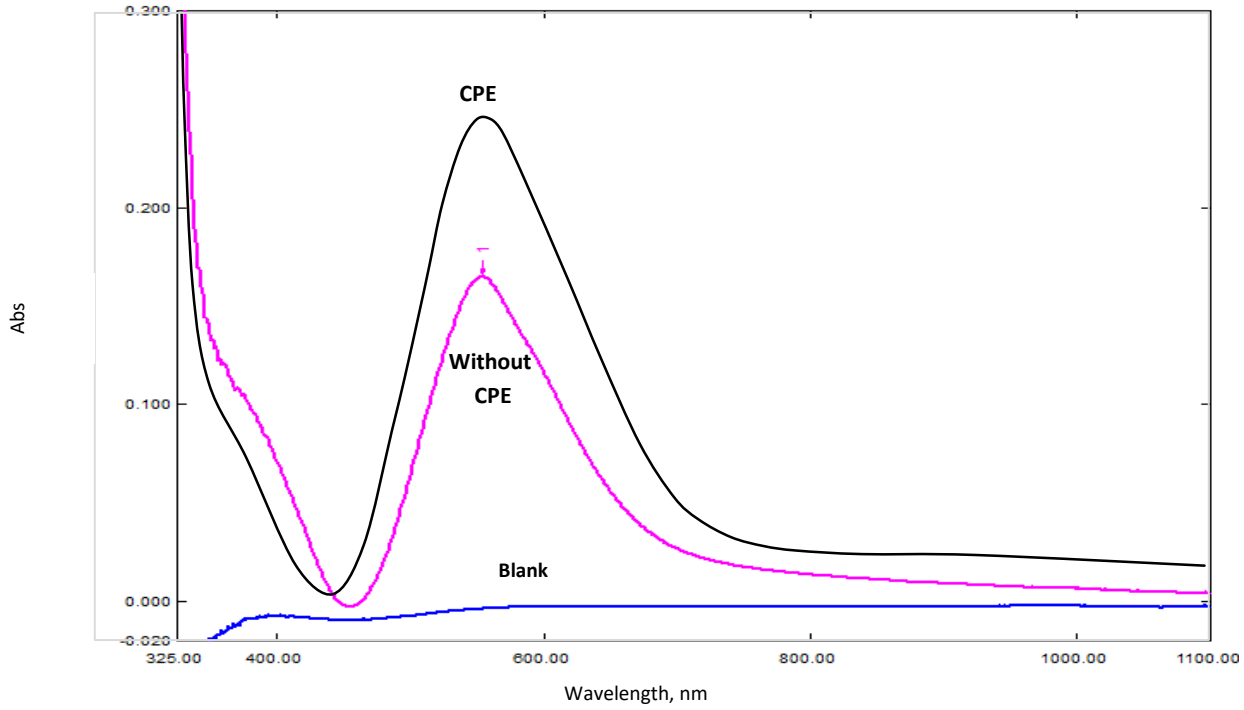
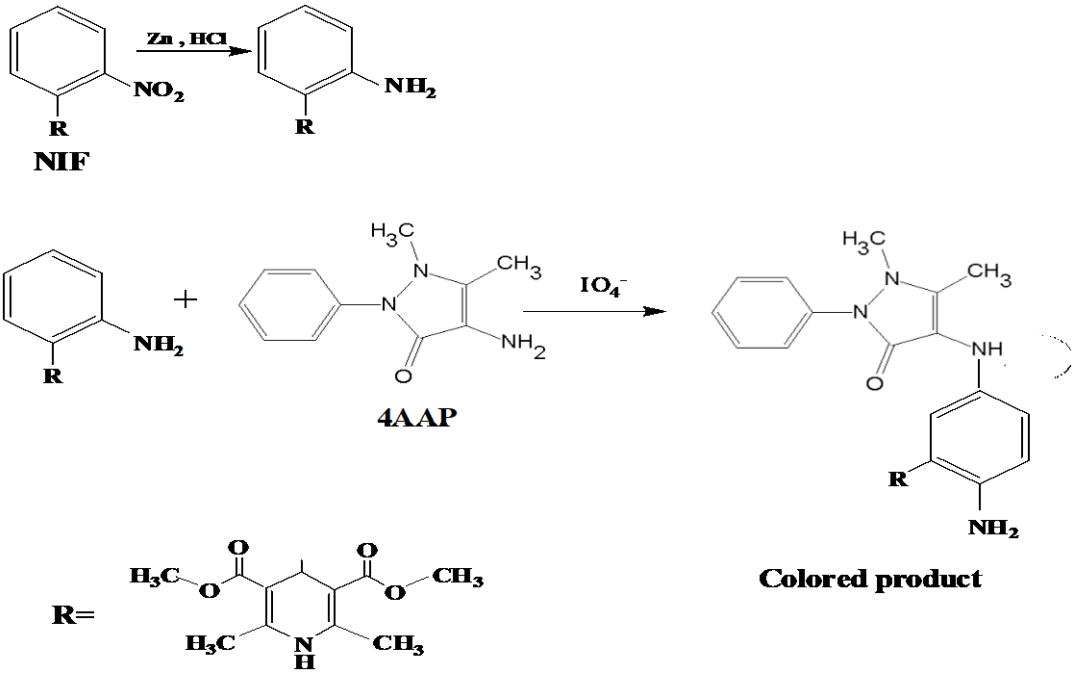


Fig. 1. The absorption spectra of the colored product and the blank were recorded with and without CPE

The proposed reaction mechanism (Scheme 1) involved two main steps: the first step is reduction the nitro group of NIF drug using zinc powder with concentrated HCl to obtain the amino group which is coupling then with 4AAP in the second step. The oxidative coupling reaction is carried out using sodium periodate as oxidant and in alkaline medium. The combining ratio was estimated by mole ratio method using equimolar concentration of both drug and reagent indicated that the complex formed between the reduced NIF and 4AAP was 1:1 respectively.



Scheme 1. Proposed reaction mechanism

Optimization of CPE method

The parameters that affecting on the efficiency of extraction and the analytical response, were studied and optimized by altering one parameter with the time and keeping the rest fixed. A solution of 300 µg of reduced NIF in a 10 mL final volume (i.e.30 µg/mL) was used in all preliminary experiments, with measuring absorbance at 554 nm against the blank.

Effect of reaction medium

The preliminary experiments showed that the reaction was carried out in alkaline medium. Several types of bases were examined but only NaOH gave the best analytical signal. In order to study the optimum volume of base added to complete the reaction a range of volumes of 0.2 M NaOH (from 0.3–2.5 mL) was added to the reactants and examined by measuring the absorbance at 554 nm. The results indicated that 1 mL of NaOH was enough for development of the reaction color product, more basic solution was decreased the absorbance (Figure 2).

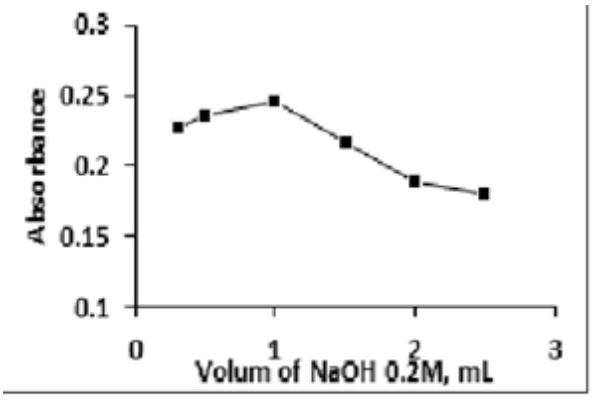


Fig. 2: Effect of volume of basic medium

Effect of 4AAP concentration

Influence of 4AAP concentration on the analytical response of colored product was examined using various range (1- 4 mL) volumes of $5x10^{-3}$ M 4AAP. The results in Figure 3 showed that the analytical signal increased with increasing 4AAP volume and reached a maximum at 3 mL then slightly decreases. As a result, the volume 3 mL of $5x10^{-3}$ M of reagent was designated as the optimal value.

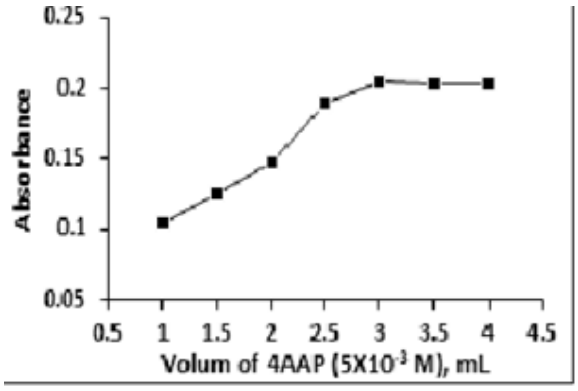


Fig. 3: Effect of 4AAP concentration

Effect of sodium periodate concentration

In order to success the oxidative coupling reaction, a strong oxidant must be available to oxidize the reagent to more reactive spices. Sodium periodate is a good oxidizing agent, freely soluble in water, cost effective and gave the best response and so it was used in the present work. Different volumes of oxidant were examined in the range (0.5-4 mL) of (0.1 M) $NaIO_4$. The results referred that the best response was maintained using 1.5 mL $NaIO_4$, and it was used in all the next experiments (Figure 4).

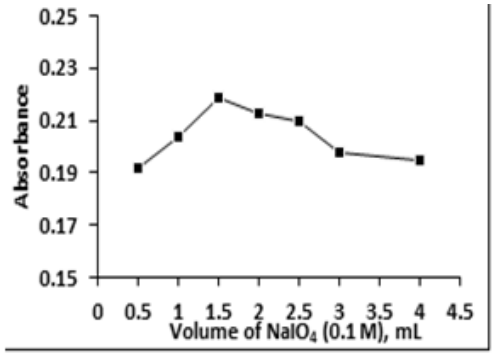


Fig. 4: Effect of volume of sodium periodate

Effect of concentration of Triton X-114

According to several experiments by using different types of surfactants, the results indicated that Triton X-114 is the best for extraction the dye. The impact of the concentration of Triton X-114 on the response of the extracted dye was established by taken a range of surfactant volumes (1-5 mL) of 4% (v/v) Triton X-114. The results (Figure 5) showed that a 3 mL of 4% Triton X-114 gave the best analytical signal with high extraction efficiency, therefore it was selected as optimum volume.

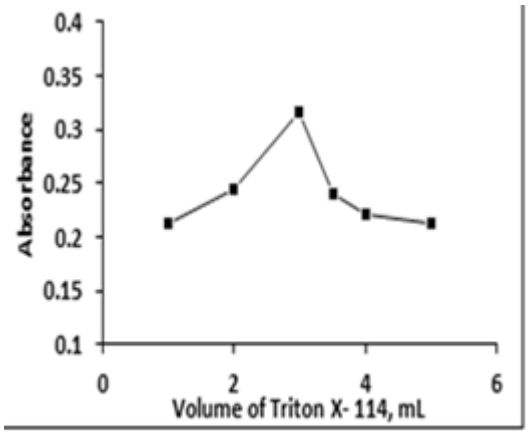


Fig. 5: Effect of volume of surfactant

Effect of temperature and time of extraction

The incubation temperature and time are essential variables which must be optimized to achieve an efficient separation of two phases with high preconcentration. Different range of incubation temperature was experimented between 25 and 80 ºC (using incubation time 20 min). Maximum analytical signal was obtained at 65 ºC (Figure 6), and this temperature was selected for the following experiments.

In addition, an incubation time need to reach to the equilibrium between two phases was also studied. The effect of this variable on extraction efficiency and absorbance of complex was studied in the range of 5-40 min at 65 ºC.

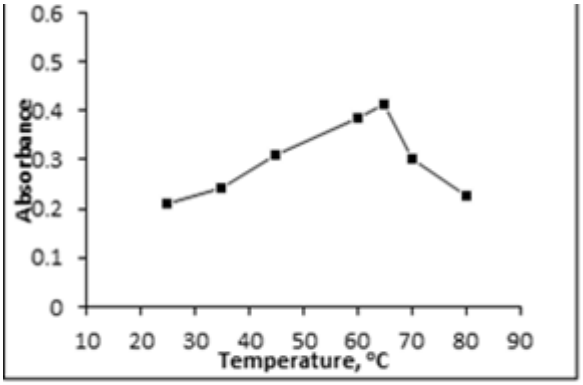


Fig. 6: Effect of temprature

An incubation time of 15 min gave maximum response and was selected as optimum time (Figure 7). Also, the 5 min time required for separation two phases using the centrifuge, was sufficient for complete separation and no considerable enhancements were detected for longer time.

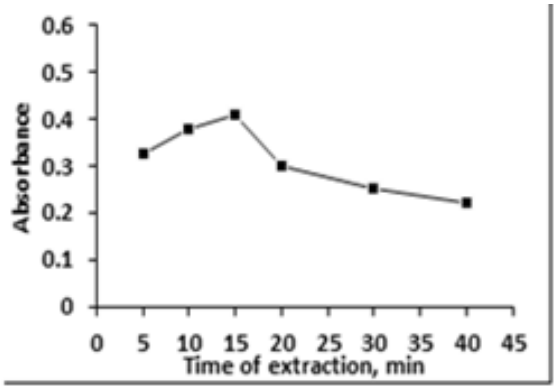


Fig. 7: Effect of time of extracion

Analytical characteristics

Using all the previous optimum conditions for assay of NIF, the calibration graph was estimated, and the linearity was in the range 0.8-45 µg mL^{-1} of NIF solution. Table 1 listed the analytical figure of merits such as linear range, regression equation, slope, intercept, and preconcentration factors. The detection limit (DL) and quantification limit(QL) were estimated according to the same guidelines: DL=3.3σ/s and QL=10σ/s (σ is the standard deviation of 10 reagent blank, and s is the slope of the calibration curve). In addition, the small values of standard deviation of the residual ($S_{y/x}$), slope (S_b), and intercept (S_a) indicated the precision of the proposed method. In addition, the results obtain from proposed CPE method were compared with batch method (without extraction). The preconcentration factor was estimated by dividing the volume of aqueous phase by the final volume of the preconcentrated phase, and it was equal to 20, whereas the enrichment factor value was equal to 2 (estimated by divided the slope of the calibration graph of CPE method to the slope of the calibration graph without CPE).

Table 1. Analytical characteristics of proposed CPE method

Parameter	With CPE	Without CPE
Regression equation	y = 0.011x + 0.0547	y = 0.005x + 0.0779
Correlation coefficient, r	0.9976	0.9978
Linearity percentage, % r2	99.52	99.56
Dynamic range (µg/mL)	0.8-45	3.0 – 80
Molar absorptivity, ε (L/mol cm)	3809.69	1731.68
Sandell sensitivity (µg/cm2)	0.09091	0.2000
Slope, b (mL/µg)	0.011	0.005
Intercept, a	0.0547	0.0779
Sy/x	0.0127	0.0095
Sb	0.0002	0.0001
Sa	0.0065	0.0049
Preconcentration factor	20	---
Enrichment factor	2	---
DL (µg/mL)	0.395	1.030
QL (µg/mL)	1.317	3.432

Accuracy and precision

In order to access the accuracy and precision of CPE method, NIF solutions of three different concentrations were analyzed under optimum conditions in three replicates. Table 2 summarized the analytical results attained from this investigation. A small values of the percentage relative error (R.E%) and relative standard deviation (R.S.D%) indicate the highly precision and accepted repeatability of the present method.

Table 2. Accuracy and precision of the proposed method

Sample	CPE			
	Conc. NIF (µg/mL)		Recovery (%) *	RSD (%) *
	Taken	Found		
1	5	4.98	99.63	2.68
2	10	10.76	107.61	1.14
3	20	20.67	103.35	1.24

*Average of three determinations.

Analysis of NIF in pharmaceutical formulations

The suggested CPE method has been applied to assay pharmaceutical tablets containing NIF. Three concentrations with six replicates were analysis under the suggested method and the recoveries values were obtained. The results are given in Table 3 indicated the good accuracy of the method and applicability of use CPE in routine estimation of NIF. To evaluate the competence of the method, the obtained results were compared with the results of UV method which selected as a comparative method [15]. For this purpose, two common tests (t-and F-tests at confidence level of 95%) were statistically used for comparison [16]. The results (t and *F*-values was not surpassed the theoretical values) pointed to insignificant difference considerable between each method (suggested and standard methods) in accuracy and precision.

Table 3. Application the proposed method in assay of NIF in dosage forms

Dosage form	Suggested method					Official method
	Taken conc. (µg/mL)	Found conc. (µg/mL)	Recovery (%) *	Mean recovery (%)	RSD (%) *	Mean recovery (%) *
Adalat® Tablet (30 mg NIF)	10	10.09	100.89	99.18	1.50	101.03
	20	19.66	98.30		1.29	
	30	29.20	97.34		1.74	
Pure NIF				101.70		101.30
F (161.447) **	89.947					
t (4.303) **	0.576					

Conclusion

The present work involved suggested an easy and very sensitive ecofriendly method for extraction and estimation of NIF in bulk and pharmaceutical forms. As compared with other methods, the method validation offered an excellent result such as linearity, high sensitivity in addition to acceptable precision. The method is safe and inexpensive and could be performed within 20 min. The suggested method could be useful for routine quality control assay of NIF in pure and pharmaceutical dosage forms (tablets).

CONFLICT OF INTEREST AND FUNDING SUPPORT

There is no conflict of interest or funding support

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