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ORIGINAL ARTICLE

Spectrophotometric method for the determination of amlodipine besylate in pure and dosage forms using 7,7,8,8-tetracyanoquinodimethane and tetracyanoethylene

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7,7,8,8-
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Abstract Two simple, rapid and sensitive spectrophotometric methods for the determination of amlodipine besylate (ADB) in pure form and in pharmaceutical preparations have been developed. The methods are based on the charge transfer reactions between the drug as electron donor with 7,7,8,8-tetracyanoquinodimethane (TCNQ) and tetracyanoethylene (TCNE) as π-acceptors in order to the formation of charge transfer (CT) complexes. These reactions give colored products which have maximum absorption bands at 745 and 396 nm for TCNQ and TCNE, respectively. Beer's law is obeyed in the concentration ranges 20–110 μg mL^{-1} and 5–35 μg mL^{-1} for ADB using TCNQ and TCNE reagents. The molar absorptivities are 2.73×10^3 and 6.43×10^3 L mol^{-1} cm and the Sandell (*S*) sensitivities are 0.14 μg cm^{-2} and 0.063 μg cm^{-2} using TCNQ and TCNE reagents, respectively, which indicate the high sensitivity of the proposed methods. The relative standard deviations (R.S.D.: 0.94 and 0.73) obtained using TCNQ and TCNE reagents, respectively, refer to the high accuracy and precision of the proposed method.

1. Introduction

Amlodipine besylate (ADB) is 2-[(2-aminoethoxy)-methyl]-4-2-chlorophenyl), 1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid, and 3-ethyl-5-methyl ester. ADB, a calcium channel blocker with vasodilatory activity similar to those of nifedipine, is mainly used in antianginal, antihypertensive and antiarrhythmic activities.[1]

Different analytical methods that have been reported for the determination of amlodipine including, high-performance liquid chromatography,[2–7] gas chromatography coupled with mass spectrometry,[8] high-performance thin layer liquid

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chromatography,[9,10] and fluorimetry.[11] A number of other extractive spectrophotometric methods,[12–16] have been also reported. Moreover amlodipine besylate in pharmaceutical preparations was determined using the formation of Charge transfer complexes based on the reaction of ADB with an electron acceptor named DDQ.[17]

Charge transfer phenomena were introduced by Mulliken and widely discussed by Foster to define a new type of adducts. Molecular interactions between electron donors and acceptors are generally the formation of intensely colored charge transfer complexes which have absorbance in visible region.[18] Charge transfer interactions within the formation of molecular complex involving a resonance with a transfer of charge from an electron donor (D) to an electron acceptor (A) were also showed by Mulliken.[19,20]

$$D + A \leftrightarrow D^{\cdot+} + A^{\cdot-} \quad (1)$$

Many drugs are easy to determine by spectrophotometry based on color charge transfer (CT) complexes formed between electron acceptors, either π- or δ-acceptors and drugs as electron donors either n or π donors. This paper reports simple, direct, sensitive and precise spectrophotometric methods for the determination of amlodipine via complexation with two π-acceptors, tetracyanoethylene (TCNE) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) which are known to yield charge transfer complexes with a variety of electron donors[21] and to use these reagents for the quantitative determination of the drug in its dosage forms.

2. Materials and methods

2.1. Instrumentation

All the absorption spectral measurements were made using a RAYLEIGHUV-1800 UV–Vis spectrophotometer (china) with Wavelength Range 190–1100 nm and Spectral Bandwidth of 2 nm equipped with quartz cells.

2.2. Chemicals and reagents

The reagents used were of analytical grade and solvents used were of spectroscopic grade. Pure ADB was obtained from commercial source (Sobhan Company, Rasht, Iran). Fresh solutions of TCNQ (Merck) or TCNE (Merck) (0.02% w/v) in acetonitrile (Merck) were prepared.

2.3. Standard solutions

Stock solution of pure ADB (1 mg mL^{-1}) was prepared by dissolving of 100 mg of ADB in 100 mL acetonitrile. The working standard solution (100 μg mL^{-1}) was prepared by stepwise dilutions of the stock solution with acetonitrile.

2.4. Procedures

2.4.1. Construction of calibration curve

Aliquots containing from 5 to 35 μg mL^{-1} of pure ADB for the TCNE method and 20–110 μg mL^{-1} for the TCNQ method were transferred into a series of 5 mL volumetric flasks. 1.0 mL of 0.02% TCNQ or TCNE solution was added to them and the solutions were diluted to volume with acetonitrile. The solutions were left to stand for 70 and 30 min at room temperature, afterward the absorbance of the standard solutions was measured at λ = 745 and 396 nm for TCNQ and TCNE methods, respectively. These measurements were performed against the reagent blank prepared simultaneously.

2.4.2. Procedure for dosage forms

Twenty tablets of ADB were weighed and powdered. The powder equivalent to 10 mg of ADB was weighed accurately, dissolved in least amount of acetonitrile, filtered through Whatmann filter paper No. 42 and washed with the acetonitrile solvent. Then, washing solution was diluted to 100 mL with acetonitrile in a 100 mL calibrated flask. The procedure was continued as described under general procedures.

3. Results and discussion

3.1. Spectral characteristics

When the solution of ADB (Lewis base) was mixed with the solution of π-acceptors (Lewis acid) in acetonitrile at room temperature, an intense green product was produced for the TCNQ method which had maximum absorption band at λ = 745 nm as shown in Fig. 1a and an intense yellow product

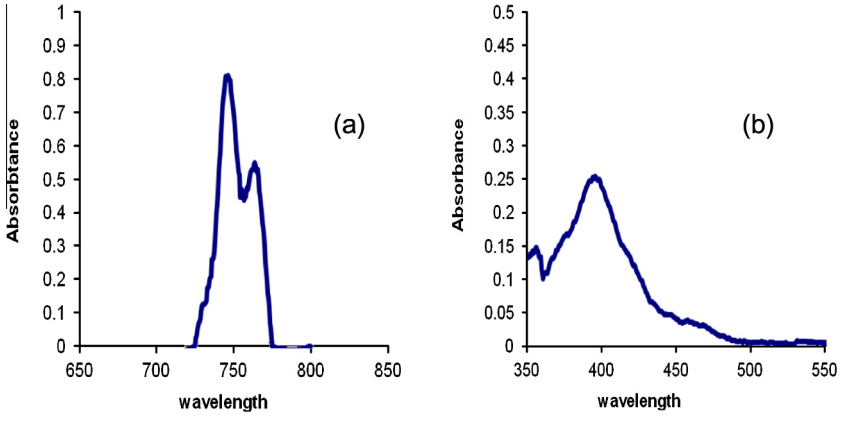


Figure 1 (a) Absorption spectra of the reaction products of ADB with TCNQ. (b) Absorption spectra of the reaction products of ADB with TCNE.

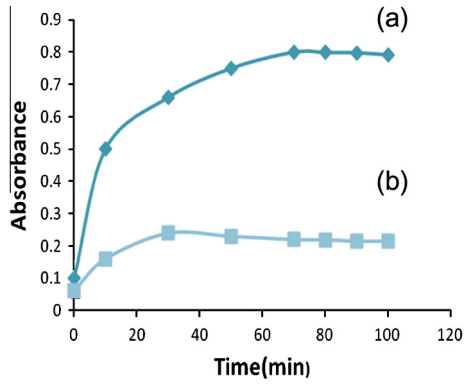


Figure 2 (a) Effect of time on the formation of colored product with ADB (50 μg mL^{-1}) and TCNQ (0.02% w/v). (b) Effect of time on the formation of colored product with ADB (30 μg mL^{-1}) and TCNE (0.02% w/v).

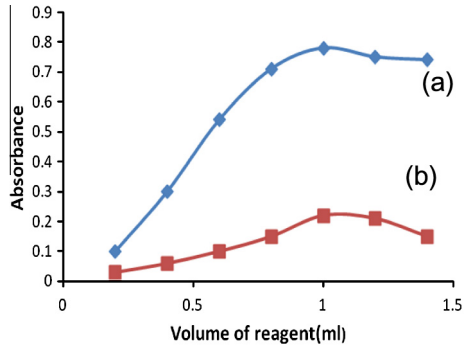


Figure 3 (a) Effect of mL reagent for the TCNQ method (ADB used in concentration of 50 μg mL^{-1}). (b) Effect of mL reagent for TCNE (ADB used in concentration of 30 μg mL^{-1}).

which exhibits a maximum absorption band at λ = 396 nm (Fig. 1b). The CT complexes were formed by the interaction of the investigated drugs as n-electron donor and TCNQ and TCNE reagents as π-acceptors.

3.2. Method optimization

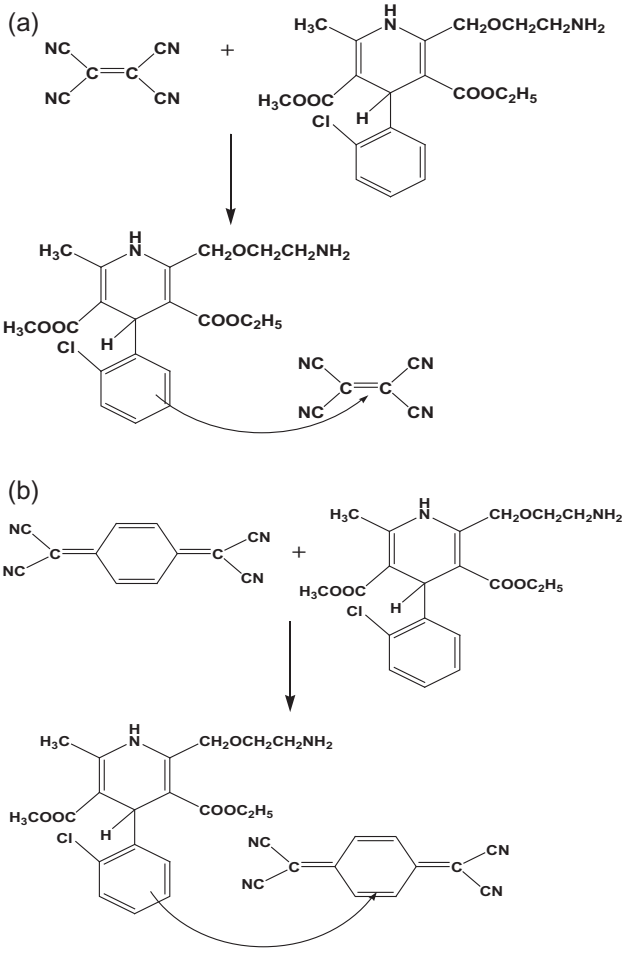
In order to optimize the conditions, we have investigated a number of parameters such as volume of reagents, nature of solvent and reaction time.

3.2.1. Effect of solvent

Different solvents were investigated in order to select the suitable solvent for TCNQ and TCNE methods. These solvents included acetonitrile, chloroform and methanol. It is found that acetonitrile is considered to be an ideal solvent for this experiment because it has a suitable solvating power for TCNE and TCNQ as well as producing more stable and reproducible absorbance. Due to the mentioned reasons we decided to complete our work with acetonitrile.

3.2.2. Effect of time

Optimization of reaction time was carried out by measuring the absorbance of the CT complexes at different time intervals at room temperature (25 °C) for both the reagents. Complete color development was attained either instantaneously or after



Scheme 1 (a) Structure of ADB–TCNE complex and (b) structure of ADB–TCNQ complex.

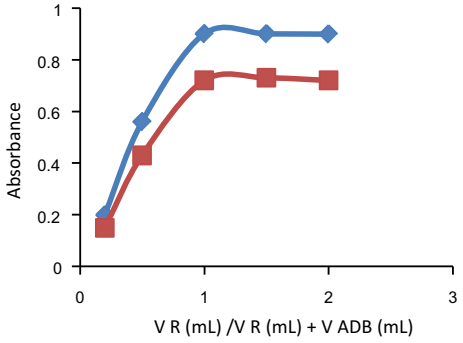


Figure 4 (a) Stoichiometry measurement through the molar ratio method for the TCNQ method (ADB and TCNQ used in the concentration of 10^{-3} M). (b) Stoichiometry measurement for the TCNE method (ADB and TCNE used in the concentration of 10^{-3} M). V_R refers to volume of reagent.

70 and 30 min for TCNQ and TCNE reagents, respectively (Fig. 2).

3.2.3. Effect of reagents volumes

Various volumes of TCNQ and TCNE reagents added to fixed concentrations of ADB in acetonitrile and the results are

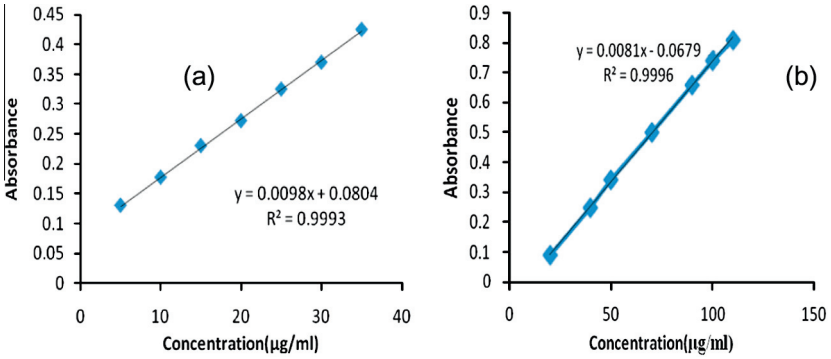


Figure 5 (a) Calibration curve for the TCNE method. (b) Calibration curve for the TCNQ method.

Table 1 Spectrophotometric parameters of determination.

Parameter	TCNQ	TCNE
λ_{max} (nm)	745	396
Beer's law limits (μg/mL)	20–110	5–35
ε (L mol^{-1} cm^{-1})	2.73×10^3	6.43×10^3
Regression equation	$Y = 0.0081X - 0.0679$	$Y = 0.0098X + 0.0804$
Correlation coefficient (r)	0.9996	0.9993
Relative standard deviation (% *RSD*)	0.94	0.73
Limit of detection (μg mL^{-1})	0.75	0.60
Limit of quantification (μg mL^{-1})	2.50	2.00
Sandell sensitivity (μg cm^{-1})	0.14	0.063

shown in Fig. 3. We found that 1 mL of 0.02% (w/v) TCNQ and TCNE is the optimized volume for the formation of CT complexes in order to cause maximum and reproducible color intensity.

3.3. Mechanism of reaction between ADB with TCNQ and TCNE

A π–π* charge transfer complex is formed by the transfer of electron from the benzene ring (electron rich group) of the ADB as electron donor to the electron-acceptor reagents (TCNE and TCNQ).[22,23] The structures of the complexes formed between the drug and reagents (TCNE and TCNQ) are shown in Scheme 1a and b.

3.4. Stoichiometry of the CT complexes

The molar ratio method is applied in order to determine the suitable ratio between ADB and TCNQ or TCNE reagents. The results show that 1:1 complexes were formed between the drug and TCNQ, TCNE reagents as shown in Fig. 4.

3.5. Method validation

3.5.1. Linearity and range

After the optimization of different parameters such as solvents, time and reagent volumes, the calibration curves can be constructed by plotting absorbances versus concentrations (Fig. 5).

Table 2 Spectrophotometric microdetermination of ADB in different pharmaceutical preparations via their reaction with TCNQ and TCNE by the proposed and reference method.[24]

Pharmaceutical preparations	Conc. taken (μg mL^{-1})	Conc. found (μg mL^{-1})	TCNE method		Reference method	
			Recovery[a] (%)	RSD[a] (%)	Recovery[a] (%)	RSD[a] (%)
Amlodipine	30.00	30.125	100.40	0.57	100.13	0.73
Amlopres	30.00	29.875	99.50	0.58	99.98	0.51

Pharmaceutical preparations	Conc. taken (μg mL^{-1})	Conc. found (μg mL^{-1})	TCNQ method		Reference method	
			RSD[a] (%)	Recovery[a] (%)	RSD[a] (%)	Recovery[a] (%)
Amlodipine	80.00	79.20	0.29	99.00	0.73	100.13
Amlopres	80.00	79.63	0.57	99.60	0.51	99.98

In the HPLC method reference parameters are as follows: C18 column 250 mm × 4.6 mm (5 μm), with acetonitrile as mobile phase:70 mM potassium dihydrogen orthophosphate buffer:methanol (15:30:55) and pH adjusted to 3.0.

[a] Average of three independent analyses.

Table 3 Standard addition determination of amlodipine in dosage forms.

Formulation name	Labeled amount (mg)	TCNQ method			TCNE method		
		Found (mg)	Recovery (%)	R.S.D[a] (%)	Found (mg)	Recovery (%)	R.S.D[a] (%)
Amlodipine	10.00	10.08	100.8	0.61	9.8	98.00	0.52
Amlopres	10.00	9.91	99.1	0.73	10.6	100.6	0.65

[a] Average of three determinations.

Beer's law is obeyed over the concentration ranges 20–110 μg mL^{-1} for TCNQ and 5–35 μg mL^{-1} for TCNE. Table 1 shows the different analytical parameters obtained such as regression equation, molar absorptivity and Sandell sensitivity. The small value of Sandell sensitivity and the high value of molar absorptivity (ε) indicate the high sensitivity of the proposed method for the determination of ADB.

3.6. Application of the proposed method

The proposed methods were successfully applied for the determination of ADB in tablet forms and the results are given in Table 2. Recovery studies were applied in order to investigate the reproducibility of the methods. The precision is expressed as the Relative Standard Deviation (R.S.D) and the data were found with three independent analysis using calibration curves. Table 2 demonstrates the low value of R.S.D as well as suitable recovery percentages which are close to 100%. The comparison of recovery percentages and R.S.D values of the proposed method and reference method[24] indicate good accuracy and precision of the proposed methods.

In addition for further investigation the amount of ADB in tablet form was obtained by the standard addition method. Table 3 demonstrates the results of standard addition determination of ADB. In the standard addition method the concentration of an unknown solution is obtained by adding different volumes of standard solution and constructing the concerned graph. Table 3 also shows that recovery percentages are close to 100% too and ranged from 99.10 to 100.80 which indicate the high efficiency of the proposed methods.

4. Conclusions

The data given above reveal that the proposed methods are simple, rapid, accurate, precise and economical. With these methods, one can do the analysis with pace at low cost without losing accuracy. The proposed methods may be applied for routine analysis as well as quantitative determination of the ADB in quality control laboratories in pharmaceutical formulations.

5. Conflict of interest

None declared.

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