Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Development of Spectrophotometric Method for the Determination of Catecholamines in Pure and Pharmaceutical Formulations, Using Pyromellitic Dianhydride Reagent by Charge Transfer Reaction

Adar Jamal Faris[1]*, Mahmood Ali Hasan[2]

[1] *Chemistry Department, Faculty of Science, University of Zakho, Kurdistan Region, Iraq*

[2] *Department of Chemistry, Faculty of Science, University of Zakho, Kurdistan Region, Iraq.*

Abstract

A novel, easy, quick and precise spectrophotometric technique has been established for the evaluation of catecholamines (methyldopa, levodopa, dopamine and carbidopa) in pure forms and in their pharmaceutical preparations, this technique is focused on the reaction of these catecholamines as n donor with pyromellitic dianhydride as π acceptor in the presence of sodium hydroxide. The maximum absorptions at 300, 302, 301, and 298 nm for methyldopa, levodopa, dopamine and carbidopa respectively. In a concentration range of Beer's law was obeyed 0.2-40, 0.2-40, 0.2-50 and 0.2-100 µg.mL^{-1} with molar absorptivities 9921.2975, 8982.8334, 6430.8797 and 5022.7469 L/mol.cm for the catecholamines respectively. The accuracy range between 98.843% and 100.891% and RSD% is less than 2% for the above catecholamines respectively. The detection limit (LOD) was 0.0518, 0.0581, 0.0638 and 0.0579 µg.mL^{-1} and limit of quantification (LOQ) was 0.1571, 0.1762, 0.1935 and 0.1756 µg.mL^{-1} for the above catecholamines in turn. The suggested method was successfully used to the analysis of catecholamines in pure as well as medications formulation with a good average recovery.

Keywords: Charge transfer reaction, catecholamines, spectrophotometric method

1. Introduction

Mulliken and Pearson created the theory of charge-transfer CT reactions [1] and Foster then carefully examined it [2]. Moving from an electron donor to an electron acceptor molecule, part of the electronic charge is transferred in charge-transfer reactions [1]–[3]. The production of charge-transfer complexes served as a foundation for the establishment simple, fast, and accurate techniques for the quantitative measurement of medications in bulk as well as/or pharmaceutical dosage forms [4]–[11].

Catecholamines are aromatic vicinal diols having amines connected to a benzene ring with 2 hydroxyl groups (catechol) (Figure 1) [12], [13].

Methyldopa (MD), a catecholamine that is frequently used as an antihypertensive drug, is a chemical compound which is also referred as α-methyl-3,4-dihydroxyphenylalanine (Figure 2). The MD only acts as a centrally located alpha-2-adrenoreceptor agonist, reducing sympathetic tone as well as blood pressure [14]. Levodopa (L-Dopa) chemically known as 3-(3,4-dihydroxyphenyl)-L-alanine (Figure 3), is an essential neurotransmitter, which has been utilizes for the healing of neural disorders including Parkinson's illness. The illness is caused by a severe decrease of dopamine [15]. L-Dopa is absorbed via the bowels and transformed to dopamine by decarboxylase after it been taken orally. As a result, levodopa can relieve Parkinson's illness symptoms while also reducing muscle rigidity, oculogyric crises, as well as tremor. However, high doses of dopamine can produce side effects include nausea, vomiting and heart arrhythmias. Dopamine (DA) chemical formula is 4-(2-aminoethyl)benzene-1,2-diol hydrochloride (Figure 4), it is a catecholamine neurotransmitter that is extensively dispersed for message transmission in the central nervous system [16], [17]. It is utilized to treating

_____

cardiogenic as well as endotoxic shock [18]–[20]. Carbidopa chemically known as (3,4-dihydroxy-benzyl)-2-hydrazinopropionic acid (Figure 5), has been utilized as a decarboxylase inhibitor. By combining levodopa and carbidopa, the dopamine concentration is effectively regulated at an acceptable level with minimal adverse effects [21]. It is essential to adjust the amount of levodopa and carbidopa in pharmaceutical dosage forms in order to produce a greater therapeutic effect and reduced toxicity.

Pyromellitic dianhydride (PMDA) is an organic molecule having the formula $C_6H_2(C_2O_3)_2$ (Figure 6). The double carboxylic acid anhydride is utilized to make polyimide polymers like Kapton. It is a white, hygroscopic solid [22].

Several strategies have been used for the evaluation of catecholamines, such including fluorometry [23], [24], capillary electrophoresis [25], [26], chromatography [27], [28], and electrochemical detection [29]. These techniques are difficult to use since they need derivatization, despite their accuracy, they have a number of drawbacks, including being time consuming, expensive, and requiring skilled operators. However, due of its intrinsic simple, economical and wide availability in laboratories for quality control, spectrophotometry is definitely the most practical analytical method for regular analysis.

The studied drugs are excellent n-electron donors and create CT complexes with π-acceptors like as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 7,7,8,8-tetracyanoquinodimethane (TCNQ), p-chloranilic acid (p-CLA) and bromanil [30]–[35] . Accordingly, the objective of the current study was to estimate a basic, direct, precise and accurate spectrophotometric technique for concurrent evaluation of methyldopa, levodopa, dopamine and carbidopa, via complexation with pyromellitic dianhydride π -acceptor in both pure as well as dosage forms.
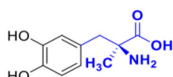

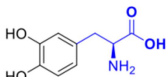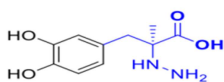**Figure 1.** Catechol   **Figure 2.** Methyldopa   **Figure 3.** Levodopa

Figure 4. Dopamine.HCl

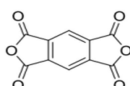**Figure 5.** Carbidopa   **Figure 6.** Pyromellitic dianhydride

_____

2. Experimental

2.1. Apparatus

All spectrophotometric measures are performed using a Perkin-Elmer, Lambda 25 UV-visible double beam spectrophotometer with matching 1-cm quartz cells. The solutions are heated on a water bath GFL 1003 Germany. Weighting is carried out on a sensitive balance type of A&D (GR-200) balance with four digitals. The pH measurements were made by utilizing Mettler Toledo FE20-Basic pH meter with integrated glass electrode.

2.2. Reagents

all substances utilized are of the best quality available, methyldopa, levodopa, dopamine, pyromellitic dianhydride and borax were supplied from (Sigma-Aldrich, China), carbidopa (Sammara drug industries, Iraq), sodium hydroxide (Alfa Aeser, Germany), potassium hydroxide and carbonate (ALPHA CHEMIKA, India), dimethyl sulfoxide, Tween-80 and ammonia solution (Merck, Germany), acetone and phosphate (ROTH, Germany), methanol (THOMAS BAKER, India), hydrochloric acid and ethanol (Scharlau, Spain), cetryltrimethylammonium bromide and sodium dodecyl sulfate (Sigma-Aldrich, Germany) and acetonitrile (BDH, England).

Pyromellitic dianhydride solution 1×10^{-3} M: It was newly prepared by dissolving 0.0218 g of PMDA in distilled water and diluting it to the proper concentration in a volumetric flask of 100 mL. This mixture was prepared every day and utilized right away.

Standard solutions of catecholamine drugs (100 µg.mL^{-1}): were prepared freshly by dissolving 0.01 g of (MD, L-Dopa, DA and Carbidopa) in distilled water and diluting it to the proper concentration in a volumetric flask of 100 mL. These solutions were prepared daily and used immediately.

Sodium hydroxide solution 2×10^{-1} M: It was prepared by dissolving 0.7999 g of sodium hydroxide in distilled water of 100 mL volumetric flask.

Sodium hydroxide solution 1.0 M: was prepared by dissolving 3.9999 g of sodium hydroxide in distilled water of 100 mL volumetric flask.

Surfactant solutions (0.1 %): were prepared by dissolving of 0.1 g of various surfactants (positive) cetyltrimethylammonium bromide (CTAB), (negative) sodium dodecyl sulfate (SDS) and

(neutral) Tween-80 in distilled water of 100 mL volumetric flask.

2.3. General Procedure

Aliquots of standard solutions of MD, L-dopa and DA (100 µg.mL^{-1}) were placed into a series of 10 mL volumetric flasks. After that, 1 mL of 1×10^{-3} M PMDA and 1 mL of 0.2 M sodium hydroxide were added and the contents were mixed well as well as diluted to the mark with distilled water. The absorbance at λ_{max} was measured against a reagent blank. The same technique was applied for carbidopa using 0.3 mL of 1×10^{-3} M PMDA and 1 mL of 1.0 M NaOH.

2.4. Procedure for the Assay of Catecholamines in Pharmaceutical Preparation

Tablets

Ten tablets were weighed and powdered finely, an accurately weight portion (250 mg of methyldopa or levodopa) was taken and dissolved in a hot distilled water. Filtration was utilized to clarify the solutions,

and suitable dilutions were prepared, and lastly treated as described in the proposed methods.

Injection

Three dopamine injection containers were thoroughly shaken and exactly a solution of measured volume, equal to 200 mg of dopamine was transported into a 100 mL volumetric flask as well as diluted with distilled water to the appropriate concentration.

3. Results and Discussion
3.1. Absorption Spectra

Catecholamines form a pale yellow charge transfer complexes with PMDA reagent in the presence of NaOH, having maximum absorptions at 300, 302, 301 and 298 nm for MD, L-dopa, DA and carbidopa respectively. These wavelengths were utilized in all subsequent measurements. (Figure 7) shows the absorption spectrum of the reaction products.
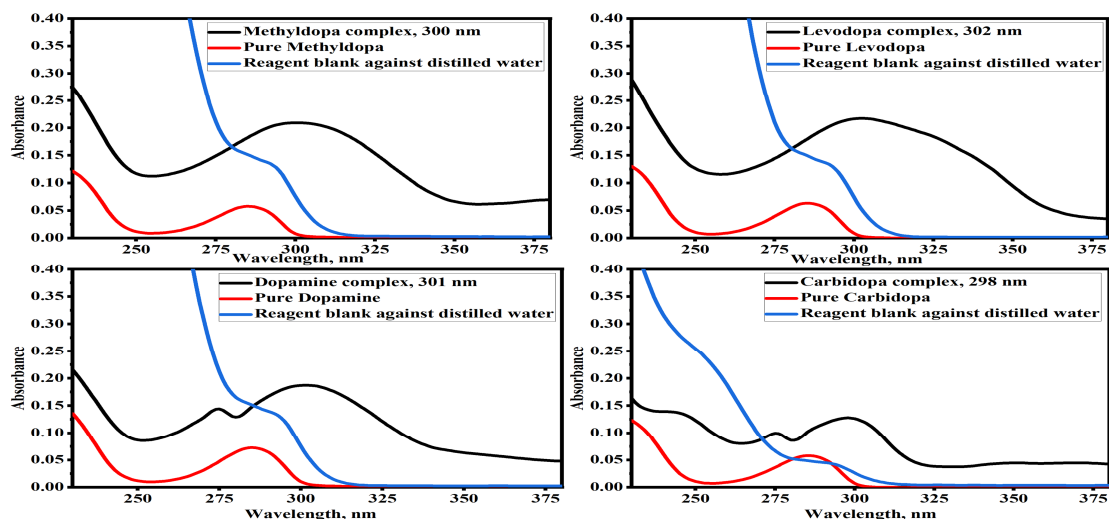

Figure 7: Absorption spectra of MD, L-Dopa and DA (4 µg.mL^{-1}), and carbidopa (5 µg.mL^{-1}) complex with PMDA (1×10^{-3} M) in the existence of 0.2 M sodium hydroxide, and 1.0 M NaOH for carbidopa against reagent blank, and reagent blank against distilled water, below optimum circumstances.

3.2. Optimum Reaction Conditions

In order to improve the suggested spectrophotometric technique, different parameters affecting the reaction such including effect of solvent, pH and buffer solution, reagent concentration, order of addition, reaction time and temperature and surfactants.

3.2.1. Effect of Solvent

The development of a charge transfer complex between donor and acceptor can be affected by the nature of the solvent. Thus, to obtain the greatest reaction conditions, solvent nature has been optimized. Various solvents including distilled water, ethanol, methanol, acetone, acetonitrile and dimethyl sulfoxide were examined, to achieve the greatest

sensitivity and product stability, it was observed that utilizing distilled water as solvent for catecholamines and PMDA reagent in the existence of NaOH as well as dilution with distilled water gave maximum colour intensity and maximum absorbance.

3.2.2. Effect of pH and Buffer Solution

The influence of pH on the reaction of catecholamines with PMDA regent were studied by varying the pH values from 3 to 12.35 using HCl and NaOH solutions, it was found that the acidic pH values had negative effects. Different values of 2×10^{-1} M NaOH for MD, L-Dopa and DA and 1.0 M NaOH for carbidopa were used, it was found that pH 11.6, 11.82, 10.60 and 12.27 gave maximum absorbances for MD, L-Dopa, DA and carbidopa respectively (Figure 8). Various bases like as sodium hydroxide (NaOH), potassium hydroxide (KOH) and ammonium hydroxide (NH$_4$OH) with constant volume (1 mL) were studied. It was observed that sodium hydroxide is more effects than the others (Figure 9). To study the influence of various buffer types like as phosphate, carbonate and borax, it was revealed that buffer solutions had negative effects with pH 11.6 for MD as an example for catecholamines (Figure 10).
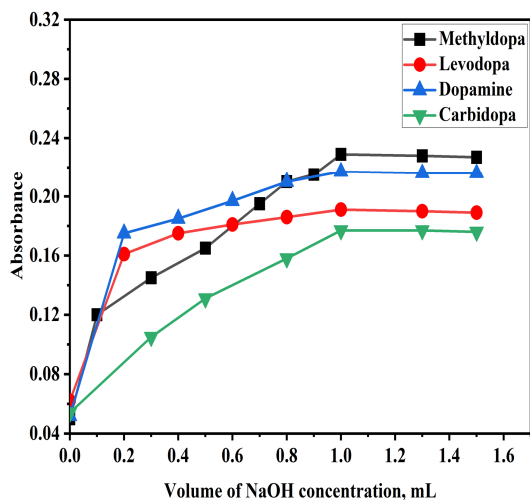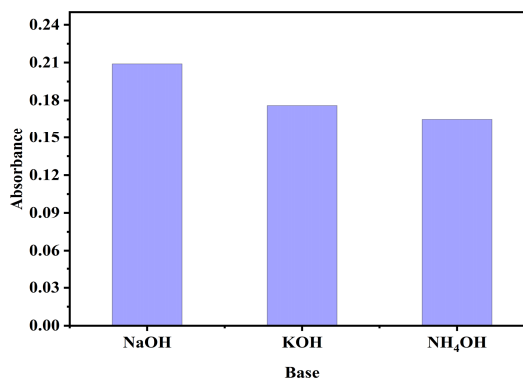
Figure 9: Bases effect on the absorption of (4 µg. mL^{-1}) MD complex with PMDA reagent
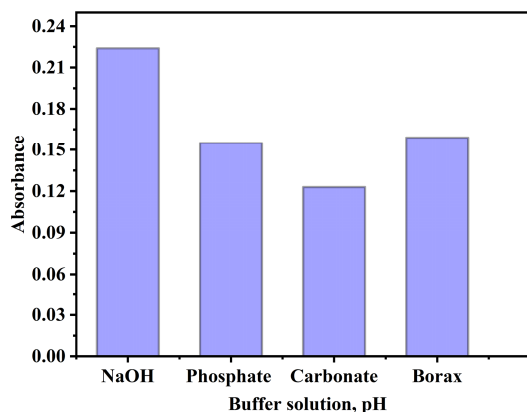
Figure 10: Effect of various buffer solutions on the absorbance of (4 µg.mL^{-1}) MD complex with PMDA reagent

3.2.3. Effect of PMDA Reagent Concentration

In order to develop the influence of the concentration of the reagent on the absorbance of the CT complexes, the effect of varying volumes (0-3.0 mL) of 1×10^{-3} M PMDA on the absorbance of solutions containing a constant amount of catecholamines in the existence of sodium hydroxide were examined. It was revealed that the absorbance increased with rising PMDA concentration & achieved maximum on utilizing 1.0 mL of for MD, L-Dopa and DA and 0.3 mL for carbidopa (Figure 11). As a result, this amount of solution was utilized in following studies.

Figure 8: Influence of sodium hydroxide on the absorption of (4 µg.mL^{-1}) MD, L-Dopa and DA, and (5 µg.mL^{-1}) of carbidopa complexes with PMDA reagent

Figure 11: Impact of amount of PMDA reagent on the absorption of (4 µg.mL^{-1}) MD, L-Dopa and DA complexes and (5µg.mL^{-1}) of carbidopa complex

3.2.4. Effect of the Order of Addition

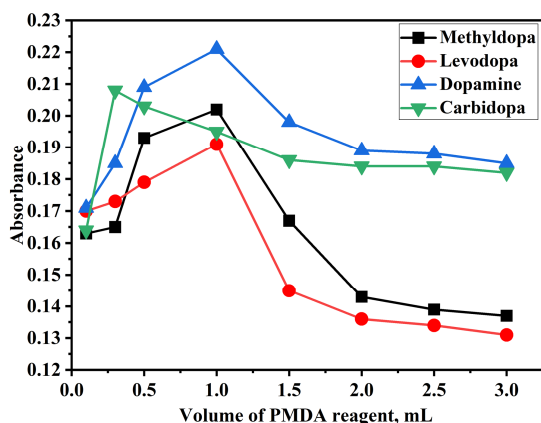In order to reach the maximum absorbance intensity, different orders of adding of reagent were studied, it was observed that the reagent addition order cited under general procedure (Reagent + NaOH + Catecholamines), was utilized in all subsequent experiments.

3.2.5. Effect of Reaction Time and Temperature

The influence of reaction time as well as temperature were studied on the creation of charge transfer complexes, by monitoring the absorbance of the products from the reaction between MD, L-dopa, DA and carbidopa and PMDA reagent at distinct times and distinct temperatures (ranges from 20-50 °C) in a controlled water bath. The results showed that the optimal reaction time were 10 min. for MD, 5 min. for L-dopa, DA and carbidopa. Maximum absorptions were obtained at 30°C for MD, 25°C for L-dopa and DA and 35°C for carbidopa (Figure 12)
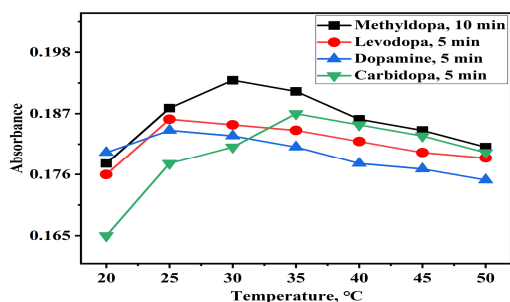

Figure 12: Temperature impact on the absorption of (4 µg.mL^{-1}) MD, L-Dopa and DA complexes, and (5µg.mL^{-1}) of carbidopa complex

3.2.6. Effect of Surfactants

A variety of surfactant types have been developed and their effects on absorbance intensity have been investigated, Cetyltrimethylammonium Bromide, Sodium Dodecyl Sulfate, and Tween-80 have been selected as cationic, anionic, and non-ionic surfactants, in turn. The findings showed that the presence of the surfactants had negative effects on the absorbance intensity. As a result, this study was abandoned.

3.2.7. Method Validation

To determine the linearity, accuracy as well as precision under ideal experimental circumstances for MD, L-dopa, DA and carbidopa. The absorbance of the complexes was measured at 300, 302, 301 and 298 nm for the above catecholamines, respectively. A linear relation was observed between absorbance & concentrations of MD, L-dopa, DA and carbidopa in the range 0.2-40, 0.2-40, 0.2-50 and 0.2-100 µg.mL^{-1} respectively. The calibration plot (Figure 13) is explained as follows:

$$Y = bX + a, \text{------------ (1)}$$

Where Y is the absorbance, the slope is b., X represents the concentration in µg.mL^{-1} & a is the intercept. Limits of Beer's Law, slope for the calibration data, intercept and correlation coefficient are summarized in (Table 1), indicating that the sensitivity of the technique. The regression equation (r^2) and associated correlation coefficient indicated that linearity existed for the MD, L-dopa, DA and carbidopa calculated by the suggested approach indicates outstanding linearity The accuracy (average recovery%) as well as relative standard deviation (RSD%) for the study of five repetitions for each of the three distinct catecholamine concentrations, it was observed that The approach is precise & accurate. Molar absorptivities were evaluated, limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the subsequent equations.

$$LOD = 3.3\sigma/b \text{ ------------ (2)}$$
$$LOQ = 10\ \sigma/b \text{ ------------ (3)}$$

Where b is the slope of the calibration plot as well as σ is the standard deviation of six calculations of reagent blanks.
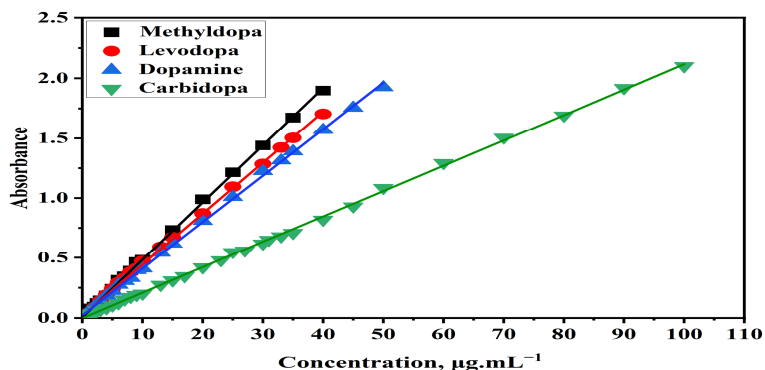
Figure 13: Calibration graphs of methyldopa, levodopa, dopamine and carbidopa

Table 1

Overview of optical properties and data for the suggested technique

Parameter	Methyldopa	Levodopa	Dopamine	Carbidopa
λ_{max}, (nm)	300	302	301	298
Limits of Beer's Law, $\mu g.mL^{-1}$	0.2-40	0.2-40	0.2-50	0.2-100
Molar absorptivity, (L/mol.cm)	9921.2975	8982.8334	6430.8797	5022.7469
LOD $(\mu g.mL^{-1})$	0.0518	0.0581	0.0638	0.0579
LOQ $(\mu g.mL^{-1})$	0.1571	0.1762	0.1935	0.1756
Sandell's sensitivity $(\mu g.cm^2)$	0.0209	0.0234	0.0257	0.0471
Correlation coefficient, r^2	0.9995	0.9992	0.9992	0.9995
Slope	0.0478	0.0426	0.0388	0.0212
Intercept	0.0068	0.0188	0.023	0.001

3.2.8. Accuracy and Precision

The precision & accuracy of the techniques were assessed by conducting five replicate studies on pure catecholamines solution at three distinct concentrations (within the workable range). Precision expressed as a percentage of relative standard deviation (RSD %), and percentage recovery (Recovery %) as accuracy of the suggested spectrophotometric techniques were evaluated (Table 2). In every case, the RSD% findings were just under 2%, showing that excellent accuracy and high precision for the suggested methods.

Table 2

Accuracy and precision data for catecholamines evaluation achieved with the suggested technique

Compounds	Amount added $(\mu g.mL^{-1})$	Recovery[*] %	Average Recovery (%)	RSD %
Methyldopa	10	98.584	98.843	0.352
	20	99.236		0.199
	30	98.708		1.031
Levodopa	10	101.347	100.891	1.515
	20	100.512		0.343
	30	100.815		0.252
Dopamine	15	101.120	100.854	1.983
	25	100.711		0.273
	35	100.730		0.432
Carbidopa	15	101.389	100.489	0.5575
	35	100.078		0.0950
	45	100.000		0.0165

*Average of five determinations

3.2.9. Stoichiometry and Stability Constant

Under the optimum conditions the stoichiometric ratio between catecholamines with PMDA reagent was found to be 1:1 (catecholamine: PMDA). Where the concentration of catecholamine constant (1.25×10^{-5} M), and the concentration of PMDA reagent are changed from $(0.5$ to $2.5) \times 10^{-5}$ M, methyldopa as an example (Figure 14).

According to the findings presented, it was estimated the seeming stability constant, by contrasting a solution's absorbance including equal amounts of each catecholamine and PMDA reagent (As) to one that contains an oversupply of PMDA reagent (Am). The average conditionally the complex's stability constant was evaluated using the follows equation.

$$K_{st} = 1-\alpha / \alpha 2C ------------- (4)$$

$$\alpha = Am-As / Am -------------- (5)$$

Where the stability constant is denoted by K_{st}, α is the degree of dissociation, and C is the complex concentration, which is equivalent to the catecholamine concentration. According to the findings in (Table 3), the complexes appear to be stable.
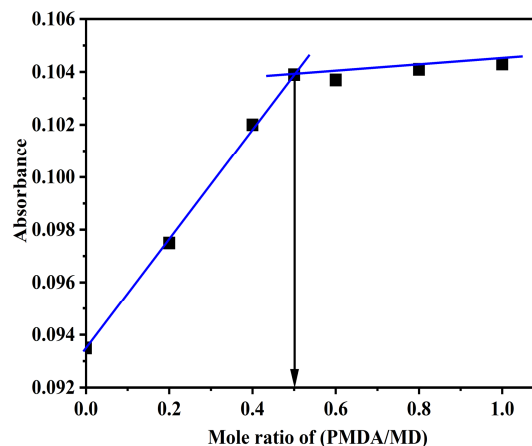

**Figure 14:** Mole ratio plot for MD-PMDA complex under the optimal conditions

Table 3

Stability constant of catecholamines – PMDA complex

| Compound | Volume, mL | Concentration, M | Absorbance | | α | Average K_{st} (L/mol) |
			A_s	A_m		
Methyldopa	0.5	0.125×10^{-4}	0.1068	0.1108	0.0361	6.995×10^{7}
	0.8	0.2×10^{-4}	0.1718	0.1762	0.0249	
	1	0.25×10^{-4}	0.242	0.2505	0.0339	
	1.5	0.375×10^{-4}	0.3678	0.3736	0.0155	
Levodopa	0.5	0.125×10^{-4}	0.1116	0.1148	0.0278	7.443×10^{7}
	0.8	0.2×10^{-4}	0.1864	0.1906	0.0220	
	1	0.25×10^{-4}	0.2309	0.2365	0.0236	
	1.5	0.375×10^{-4}	0.3495	0.3606	0.0307	
Dopamine	0.5	0.125×10^{-4}	0.0923	0.0955	0.0335	7.119×10^{7}
	0.8	0.2×10^{-4}	0.1446	0.1483	0.0249	
	1	0.25×10^{-4}	0.1804	0.1841	0.0200	
	1.5	0.375×10^{-4}	0.2618	0.2686	0.0253	
Carbidopa	0.5	0.125×10^{-4}	0.0981	0.1020	0.0382	2.415×10^{7}
	0.8	0.2×10^{-4}	0.1146	0.1295	0.1150	
	1	0.25×10^{-4}	0.1333	0.1436	0.0717	
	1.5	0.375×10^{-4}	0.2479	0.2550	0.0278	

3.2.10. Reaction Mechanism

The formation of radical ions is thought to result from the interaction of catecholamines as n-donor and PMDA as π-acceptor. Transfer of all electrons from the donor molecule to the accepting moiety may have occurred with high values of molar absorptivity [36]. Though, the suggested mechanism for the reaction is illustrated in (Scheme 1).
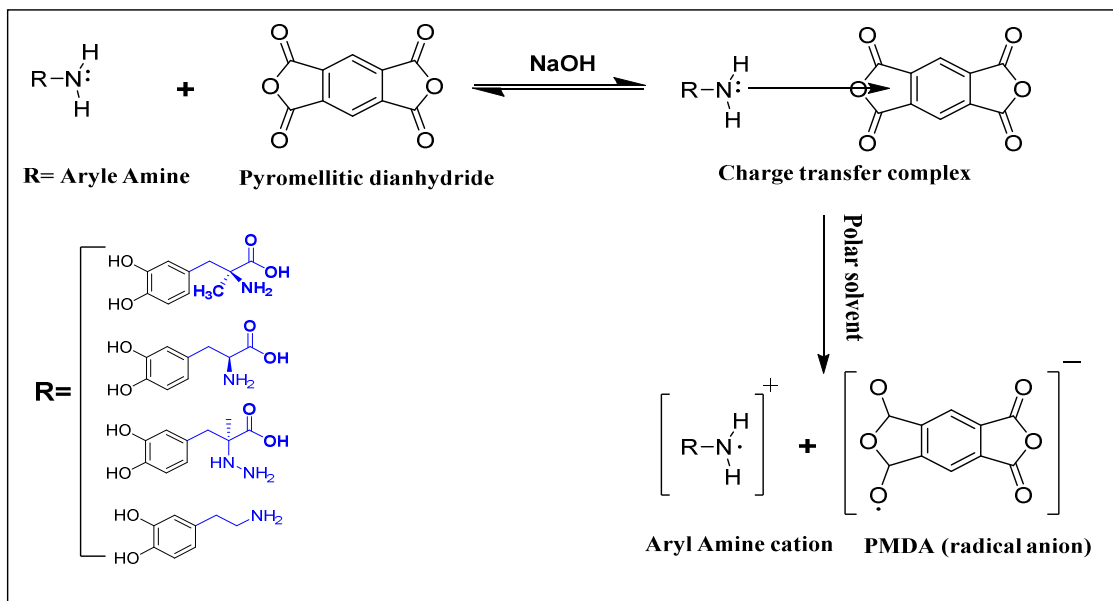
Scheme 1. Suggested mechanism for the catecholamines:PMDA charge transfer complexes.

3.2.11. Selectivity

From the values of intercepts of calibration curves of methyldopa, levodopa, dopamine and carbidopa, also from the values of the recoveries of these drugs by application of standard addition method it is appeared that, the proposed method was found to be selective for the determination of catecholamines in the presence of various excipients. It was found that the studied excipients do not interfere in the determination of catecholamines in its dosage form.

3.2.12. Analytical Application

The suggested process has been effectively used to the evaluation of methyldopa, levodopa as well as dopamine in pharmaceutical dosage form. The concentration of catecholamine drugs were determined by direct measurement on appropriate standard calibration curve (Table 4). The similar findings were obtained by utilizing the standard addition method for methyldopa tablet, levodopa tablet and dopamine injection (Table 5) and (Figure 15), by using different concentration of methyldopa and levodopa tablets and dopamine injection (3, 5 and 7 $\mu g.mL^{-1}$) with different concentration of pure drug which the range between (5-25 $\mu g.mL^{-1}$). The results showed that the technique is free from interferences.

Table 4

Assay of catecholamine drugs in pharmaceutical preparation utilizing the suggested technique

Pharmaceutical Preparation	Certified Value	Company	Amount Present ($\mu g\ mL^{-1}$)	Drug Content found	Recovery[*] (%)	Average Recovery (%)	RSD (%)
Methyldopa tablets	250 mg	Accord, UK	2 15 25	1.961 14.956 24.992	98.082 99.707 99.969	99.252	1.23 0.13 0.10
Methyldopa tablets	250 mg	SAFA, DIALA-IRAQ	2 15 25	1.963 14.967 24.988	98.152 99.781 99.955	99.296	1.61 0.11 0.09
Levodop Sinemet tablets	250 mg	MSD, Italy	2 15 25	1.986 14.867 24.799	99.334 99.118 99.197	99.216	0.33 0.04 0.01

| Levodopa Bidopa tablets | 250 mg | Pioneer, Iraq | 2 | 1.978 | 98.943 | | 0.88 |
			15	14.887	99.248	99.072	0.11
			25	24.756	99.025		0.01
Dopamine.HCl Dopasel Injection	200 mg	OSEL, Turley	2	1.988	99.428		0.31
			15	14.954	99.696	99.606	0.15
			25	24.923	99.694		0.25

*Average of five evaluations

Table 5

Assay of catecholamine drugs in pharmaceutical preparation utilizing standard addition method

Pharmaceutical Preparation	Company	Drug taken ($\mu g\ mL^{-1}$)	Recovery* (%)	RSD (%)
Methyldopa tablets	Accord, UK	3	100.149	0.159
		5	100.003	0.084
		7	100.823	0.466
Methyldopa tablets	SAFA, DIALA-IRAQ	3	100.387	0.527
		5	100.845	0.911
		7	99.961	0.865
Levodopa, Sinemet tablets	MSD, Italy	3	100.684	1.109
		5	100.550	0.819
		7	100.879	0.328
Levodopa Bidopa tablets	Pioneer, Iraq	3	100.493	0.928
		5	100.446	0.948
		7	100.520	0.787
Dopamine.HCl Dopasel Injection	OSEL, Turley	3	100.644	0.581
		5	102.068	1.275
		7	102.055	0.909

*Average of five determinations
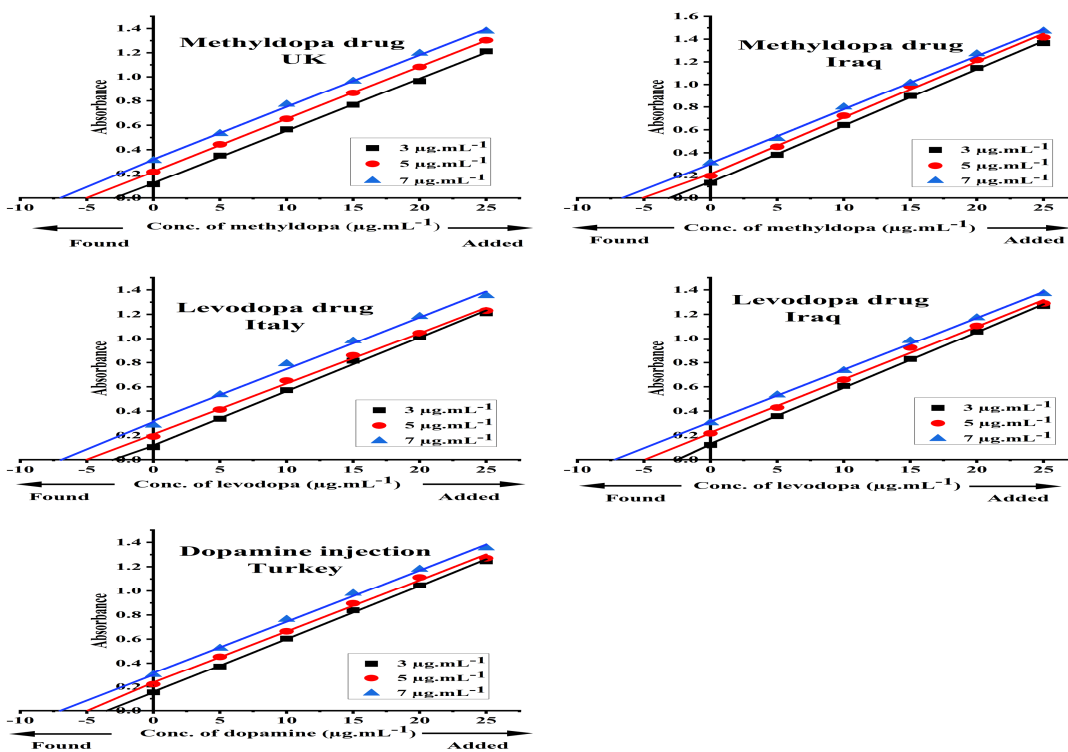
Figure 15: Standard addition procedure for the evaluation of methyldopa & levodopa in tablets, and dopamine in injection

_____

3.2.13. Comparison with Reported Method

(Table 6) shows the comparison between some of analytical variables obtained from present method with that of the recent spectrophotometric methods.

From the table, the suggested method is more sensitive than some other methods, simple and takes short time for colour development.

Table 6

Comparison of the present method with reported spectrophotometric methods

Parameters	Methods				
	PMDA	Bromanil [13]	1,10-Phenanthroline [37]	4-chloro-7-nitrobenzo-2-oxa-1,3-diazole [38]	DCQ [39]
λ_{max}, nm	300	350	510	470	400
pH	11.6	9	----	12.3	8.0
Solvent	Distilled water	Ethanol	Distilled water	Ethanol	Distilled water
Temp., ℃	30	40	80	70	Room temp.
Development time, min	10	35	40	15	1 hour
Beers law range, $\mu g.mL^{-1}$	0.2-40	1-25	0.1-2.8	1.6-17.6	4–20
Molar absorptivity, (L/mol.cm)	9921.2975	8075	1.8510×10^5	1.9337×10^4	6.42×10^3
Sandell's sensitivity, $\mu g.cm^2$	0.0209	----	3.201	----	0.1–0.6
LOD ($\mu g.mL^{-1}$)	0.0518	----	----	5.536×10^{-3}	1.1
LOQ ($\mu g.mL^{-1}$)	0.1571	----	----	----	3.21
Correlation coefficient, r^2	0.9995	0.9978	0.9998	0.9988	0.9975
Recovery (%)	98.843	101.06	99.98	----	----
RSD (%)	0.527	0.97	0.896	0.033	----

4. Conclusion

A fresh spectrophotometric technique was established for the estimation of methyldopa, levodopa, dopamine and carbidopa. The procedure is fast, easy, sensitive, reproducible and economical. The method may be suitable for routine analysis. Hence, this technique can be utilized for the evaluation of catecholamines in both pure in addition in pharmaceutical formulations.

5. Acknowledgments

6. References

[1] R. S. Mulliken, R. S. Mulliken, and W. B. Person, Molecular complexes: a lecture and reprint volume. Wiley-Interscience, 1969.

[2] R. Foster and C. A. Fyfe, "Nuclear magnetic resonance of organic charge-transfer complexes," Prog. Nucl. Magn. Reson. Spectrosc., vol. 4, pp. 1–89, 1969.

[3] K. Das, N. Sarkar, A. K. Ghosh, D. Majumdar, D. N. Nath, and K. Bhattacharyya, "Excited-state intramolecular proton transfer in 2-(2-hydroxyphenyl) benzimidazole and-benzoxazole: effect of rotamerism and hydrogen bonding," J. Phys. Chem., vol. 98, no. 37, pp. 9126–9132, 1994.

[4] R. I. El-Bagary, E. F. Elkady, and B. M. Ayoub, "Spectrophotometric methods for the determination of sitagliptin and vildagliptin in bulk and dosage forms," Int. J. Biomed. Sci. IJBS, vol. 7, no. 1, p. 55, 2011.

[5] H. Wu and L. M. Du, "Spectrophotometric determination of anilines based on charge-transfer reaction," Spectrochim. Acta Part A Mol. Biomol. Spectrosc., vol. 67, no. 3–4, pp. 976–979, 2007.

[6] I. Saito, T. Matsuura, and K. Inoue, "Formation of superoxide ion via one-electron transfer from electron donors to singlet oxygen," J. Am. Chem. Soc., vol. 105, no. 10, pp. 3200–3206, 1983.

[7] I. A. Darwish, "Analytical study for the charge-transfer complexes of losartan potassium," Anal. Chim. Acta, vol. 549, no. 1–2, pp. 212–220, 2005.

[8] H. M. Elqudaby, G. G. Mohamed, and G. M. G. El-Din, "Analytical studies on the charge transfer complexes of loperamide hydrochloride and trimebutine drugs. Spectroscopic and thermal characterization of CT complexes," Spectrochim. Acta Part A Mol. Biomol. Spectrosc., vol. 129, pp. 84–95, 2014.

[9] H. Salem, "Analytical study for the charge-transfer complexes of pregabalin," E-Journal Chem., vol. 6, no. 2, pp. 332–340, 2009.

[10] N. Z. Alzoman, M. A. Sultan, H. M. Maher, M. M. Alshehri, T. A. Wani, and I. A. Darwish, "Analytical study for the charge-transfer complexes of rosuvastatin calcium with π-acceptors," Molecules, vol. 18, no. 7, pp. 7711–7725, 2013.

[11] S. Hesham, "Analytical study for the charge-Transfer complexes of gabapentin," African J. Pharm. Pharmacol., vol. 2, no. 7, pp. 136–144, 2008.

[12] R. M. Wightman et al., "Real Time Characterization of Dopamine Overflow and Uptake in the Rat Striatum," Neuroscience, vol. 25, no. 2, pp. 513–523, 1988.

[13] M. Al-Sharook, "Spectrophotometric determination of catecholamines in

_____

pharmaceutical preparations via charge transfer complex formation using bromanil reagent," J. Educ. Sci., vol. 19, no. 2, pp. 1–11, 2007.

[14] E. A. Gadkariem, K. E. E. Ibrahim, N. A. A. Kamil, M. E. M. Haga, and H. A. El-Obeid, "A new spectrophotometric method for the determination of methyldopa," Saudi Pharm. J., vol. 17, no. 4, pp. 289–293, 2009.

[15] J. P. Remington and A. R. Gennaro, Remington's pharmaceutical sciences. Mack Pub., 1985.

[16] A. M. Othman, N. M. H. Rizka, and M. S. El-Shahawi, "Potentiometric determination of dopamine in pharmaceutical preparations by crown ether-PVC membrane sensors," Anal. Sci., vol. 20, no. 4, pp. 651–655, 2004.

[17] B. S. AYDIN and İ. Bulduk, "A Validated HPLC-UV Method for Determination of Dopamine HCl in Injectable Solutions," Eurasian J. Biol. Chem. Sci., vol. 3, no. 2, pp. 116–120, 2020.

[18] J. J. B. Nevado, J. M. L. Gallego, and P. B. Laguna, "Flow-injection spectrophotometric determination of adrenaline and dopamine with sodium hydroxide," J. Pharm. Biomed. Anal., vol. 14, no. 5, pp. 571–577, 1996.

[19] C. L. Guan, J. Ouyang, Q. L. Li, B. H. Liu, and W. R. G. Baeyens, "Simultaneous determination of catecholamines by ion chromatography with direct conductivity detection," Talanta, vol. 50, no. 6, pp. 1197–1203, 2000.

[20] J. Liu, Z.-H. Wang, G.-A. Luo, Q.-W. Li, and H.-W. Sun, "The interaction of DNA with dopamine by spectroscopic and electrochemical methods," Anal. Sci., vol. 18, no. 7, pp. 751–755, 2002.

[21] M. A. Karimi, M. H. Mashhadizadeh, M. M. Ardakani, and N. Sahraei, "Simultaneous kinetic-potentiometric determination of levodopa and carbidopa using multivariate calibration methods," J. Food Drug Anal., vol. 16, no. 5, pp. 39–47, 2008.

[22] J. B. Dunn, M. L. Burns, S. E. Hunter, and P. E. Savage, "Hydrothermal stability of aromatic carboxylic acids," J. Supercrit. Fluids, vol. 27, no. 3, pp. 263–274, 2003.

[23] R. Laverty and K. M. Taylor, "The fluorometric assay of catecholamines and related compounds: improvements and extensions to the hydroxyindole technique," Anal. Biochem., vol. 22, no. 2, pp. 269–279, 1968.

[24] J. D. Sapira, T. Klaniecki, and M. Rizk, "Modified fluorometric method for determining plasma catecholamines," Clin. Chem., vol. 17, no. 6, pp. 486–491, 1971.

[25] R. Zhu and W. T. Kok, "Determination of catecholamines and related compounds by capillary electrophoresis with postcolumn terbium complexation and sensitized luminescence detection," Anal. Chem., vol. 69, no. 19, pp. 4010–4016, 1997.

[26] Z. D. Peterson, D. C. Collins, C. R. Bowerbank, M. L. Lee, and S. W. Graves, "Determination of catecholamines and metanephrines in urine by capillary electrophoresis–electrospray ionization–time-of-flight mass spectrometry," J. Chromatogr. B, vol. 776, no. 2, pp. 221–229, 2002.

[27] H. Hallman, L.-O. Farnebo, B. Hamberger, and G. Jonsson, "A sensitive method for the determination of plasma catecholamines using liquid chromatography with electrochemical detection," Life Sci., vol. 23, no. 10, pp. 1049–1052, 1978.

[28] M. A. Raggi, C. Sabbioni, G. Casamenti, G. Gerra, N. Calonghi, and L. Masotti, "Determination of catecholamines in human plasma by high-performance liquid chromatography with electrochemical detection," J. Chromatogr. B Biomed. Sci. Appl., vol. 730, no. 2, pp. 201–211, 1999.

[29] B.-M. Eriksson and B.-A. Persson, "Determination of catecholamines in rat heart tissue and plasma samples by liquid chromatography with electrochemical detection," J. Chromatogr. B Biomed. Sci. Appl., vol. 228, pp. 143–154, 1982.

[30] O. ARMAĞAN, "Development and validation of selective spectrophotometric methods for the determination of pregabalin in pharmaceutical preparation," Chinese J. Chem., vol. 27, no. 4, pp. 781–786, 2009.

[31] M. Walash, M. S.-E. Din, M.--S. Metwalli, and M. RedaShabana, "Spectrophotometric determination of nizatidine and ranitidine through charge transfer complex formation," Arch. Pharm. Res., vol. 27, no. 7, pp. 720–726, 2004.

[32] A. Önal, E. Ş. Kepekçi, and A. Öztunç, "Spectrophotometric methods for the determination of the antidepressant drug paroxetine hydrochloride in tablets," J. AOAC Int., vol. 88, no. 2, pp. 490–495, 2005.

[33] N. Rahman and S. N. H. Azmi, "Spectrophotometric determination of amlodipine besylate by charge-transfer complex formation with p-chloranilic acid," Anal. Sci., vol. 16, no. 12, pp. 1353–1356, 2000.

[34] K. D. Reddy, S. Rao, and G. Venkateshwarlu, "Spectrophotometric Determination of Drugs Using p-Chloranilic Acid as Analytical Reagent," Asian J. Res. Chem., vol. 4, no. 8, pp. 1311–1317, 2011.

[35] S. T. Ulu, "A rapid and highly sensitive spectrofluorimetric method for the determination of venlafaxine by charge transfer complexation reaction with p-bromanil and p-chloranil," Opt. Spectrosc., vol. 110, no. 4, pp. 508–512, 2011.

[36] N. Theia'a, N. M. S. Mohammed, and P. A. Abdul-Jabar, "SPECTROPHOTOMETRIC DETERMINATION OF OXYMETAZOLINE HYDROCHLORIDE VIA CHARGE TRANSFER REACTION USING 2, 3-DICHLORO-5, 6-DICYANO-1, 4-BENZOQUINONE (DDQ) REAGENT," Eur. Chem. Bull., vol. 4, no. 8, pp. 372–377, 2015.

[37] T. Al-Ghabshaa, M. Al-Enizzi, and Z. Al-Abdaly, "Sensitive spectrophotometric method for determination of catecholamines in pure and pharmaceutical formulations," J. Educ. Sci., vol. 19, no. 3, pp. 1–12, 2007.

[38] Q. N. Rashid, M. H. Bakir, and S. O. Baban, "Spectrophotometric determination of methyldopa in pure form and in the

_____

pharmaceutical preparations," Tikrit J. Pharm. Sci., vol. 11, no. 1, pp. 67–77, 2016.

[39] E. A. Gadkariem, K. E. E. Ibrahim, N. A. A. Kamil, M. E. M. Haga, and H. A. El-Obeid, "A new spectrophotometric method for the determination of methyldopa," Saudi Pharm. J., vol. 17, no. 4, pp. 289–293, 2009, doi: 10.1016/j.jsps.2009.10.005.

_____