MICRO DETERMINATION OF DOPAMINE IN BIOLOGICAL SAMPLES BY COUPLING REACTION

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Abstract: The paper presents the development of an analytical method to determine dopamine by coupling reaction with diazotized solution of 4-Aminoantipyrine in basic media to give a compound with a single azo dye salt having orange color soluble in water. A calibration curve for a range of concentration ($9.14x10^{-5}$ – $2.28x10^{-3}$ $\mu g.mL^{-1}$) was realized and the value of molar absortivity was 1.5x103 $L.mol^{-1}.cm^{-1}$, with a relative standard deviation more than 1.26% and a recovery 98.77%. As we study the nature of the azo dye by mole fraction method, from the practical value we found that the mole fraction of the dye compound is 1:1 (dopamine: 4- amino antipyrine) and the stability factor reach to 1.4x106 $L.mol^{-1}$. The described procedure is very simple, low-time-consuming, provides high throughput of examined samples, and could be used for routine screening and confirmatory analyses as well. The method was successfully validated to the analysis of the dopamine in biological samples.

Keywords: dopamine, coupling reaction, biological samples, azo dyes, spectrophotometric.

1. INTRODUCTION

Dopamine (DA) is an important neuron transmitter compound widely used in the brain for message transfer in the mammalian central nervous system [1, 2]. Abnormal concentration levels of DA may lead to several diseases, such as Parkinson's disease [3], schizophrenia and HIV infection. Dopamine (Figure 1) is currently the subject of intense research focus by chemists and neuroscientists [4-6]. Therefore, it is of great significance to develop simple and rapid determination methods of dopamine.

The diazotizing and coupling reactions are considered one of the good, easy, fast and useful method for analytical applications for samples [4] especially the samples which are difficult to measure in their original form due to the interference that affect the determination operation. This method depends on the formation of a colored azo dye [5] which absorbs light at a specific wave length, this is considered a sensitive method of determination with a wide range of applications, for e.g.: as a diazotizing 4- amino antipyrine compound used in the determination of little amount of pyrrole in dilute solution.

This method depends on the reaction of pyrrole with diazotized compound for 4- amino antipyrine compound in the present of sodium acetate to produce a single stable azo dye soluble in water with a dark yellow color that can determine the dye absorbance at a wavelength about 420 nm [6].

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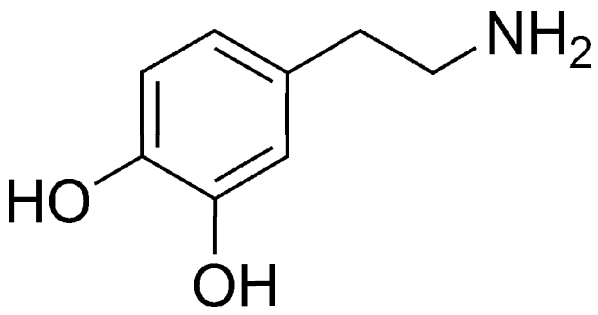

Fig. 1. The structure of dopamine.

Other examples: the determination of sulfonamide, and its coupling reaction with phenol in basic media yellow azo dye which have a maximum absorbance at 425 nm, determination of phenols in drink water [7] which depend on the reaction of nitric acid with phenol to give nitro phenol , the last compound couples with phenol to give colored product. This method have a specific properties as its easy and fast also can be applicable on substituted phenol in p-position there for we can determine some of the pharmaceutical cosmetics which have a subtitled phenols.

Several analytical methods have been developed for the analysis and determination of DA including Liquid Chromatography [8], Liquid Chromatography–Mass Spectrometry (LC-MS) [9, 10], Enzyme-Linked Immunosorbent Assay (ELISA) [11], High Performance Liquid Chromatography (HPLC) [12], and Spectrophotometry [13].

In this paper, we proposed a suitable procedure for the micro determination of dopamine, based on the coupling reaction of DA with diazotized compound of 4- amino antipyrine in basic media.

2. EXPERIMENTAL SETUP

2.1. Standard solutions

Dopamine solution (50 $\mu g.mL^{-1}$). Was prepared by dissolving 0.50 mg of DA (from BDH ANALAR company) in 50 mL absolute ethanol then completed with distilled water to 250 mL in volumetric flask.

Diazotizing solution for 4-amino antipyrine (100 $\mu g.mL^{-1}$). Was prepared by weighting the appropriated amount of compound (from BDH ANALAR) then dissolving it in 40 mL of distilled water then the solution was heated to increase the solubility, then 4 mL of 0.7 standard hydrochloric acid was added with cooling to zero degree by using an ice bath then added 0.0077 mg sodium nitrate with mixing. After 5 minute the diazonium final solution was poored in a 250 ml volumetric flask, and complete the volume with cooling distilled water (4 °C). The solution was kept in the freezer. The final solution will be stable for 1 hr in room temperature, which is (22 °C).

Hydrochloric acid from (FLUKA A.G company) (0.7 M). Was prepared by dilution of concentrated hydrochloric acid then titrate it with standard sodium carbonate to fix the concentration to (0.7 M).

Sodium carbonate Na2CO3 (0.7 M) from (BDH company). Sodium carbonate was dried for an hour in (115 °C) in watch glass then cooled and weighted 3.32449 mg that were dissolved in distilled water and brought to 250 mL in volumetric flask.

Solution (8M) of sodium hydroxide (from Fluka A.G company). Prepared from standard ammonia solution, to determine the exact morality for ammonia we prepare (8M) of NaOH according to dilution equation.

2.2. Buffer solution

We prepared buffer solutions with pH=3.2 and 6.4 respectively. The Table 1 shows the preparation of citrate buffer using Na_2HPO_4, 0.1M and citric acid 0.2M.

Table 1. Prepared buffer solution (buffer citrate).

pH	0.1 M Na_2HPO_4	0.2 M Citric acid
2.3	2.13 mL	19.65mL
7.4	18.45mL	1.59mL

We prepared buffer solutions with pH = 9.8 and 11.10 respectively. The Table 2 shows the preparation of carbonate buffer using Na_2CO_3. 0.2M and bicarbonate buffer 0.2M.

Table 2. Prepared buffer solution (carbonate buffer).

pH	0.2 M Na_2CO_3	0.2 M $NaHCO_3$
9.8	4 mL	6 mL
11.10	10 mL	2 mL

We prepared phosphate buffer solutions with pH=11.6. by taking 70 ml of (0.3 M) of Na_2HPO_4 solution and added to it 5.5 ml of (0.2M) sodium hydroxide solution then dilute to 250 ml by using distal water.

2.3. Determination of dopamine by the coupling reaction

The coupling operation done by using (50 $\mu g.mL^{-1}$) of dopamine with 4 amino antipyrine (100 $\mu g.mL^{-1}$) in the presence of phosphate buffer (pH=10.80). 3mL of diazotized compound was added to 6 mL of buffer solution then added different volumes of dopamine then added to it 50 mL distilled water after that measure the absortivity (A) for this solution against the blank solution at a wave length 449 nm and illustrated in Figure 2, as we draw calibration curve between the absorbance and the concentration we have a straight line.

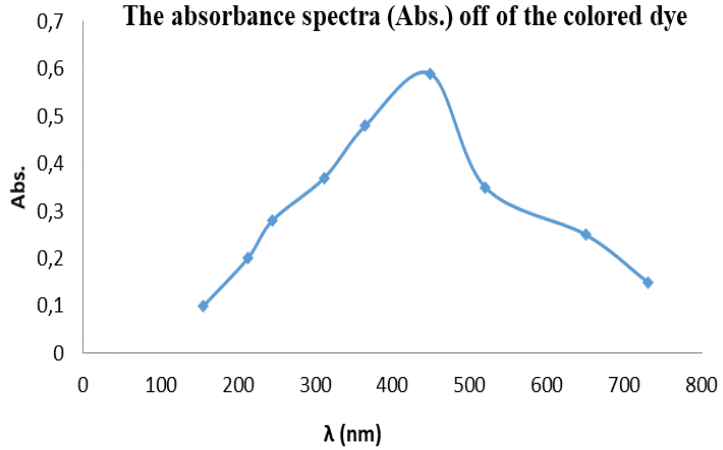

Fig. 2. The absorbance spectra of the colored product.

3. RESULTS AND DISCUSSION

3.1. Primary test

We notice that in mixing of dopamine compound with 4- amino antipyrine in basic media, a single azo dye with orange color soluble in water formed, the reaction involved two steps:

- 4-amino antipyrine compound reacted in acidic media at 0˚C with equal amount of sodium nitrate to give diazonium salt.
- When DA was added to the diazotizing compound in basic media a single azo dye with orange color formed which gave a maximal absortivity at a wave length of 449 nm.

3.2. The parameters that influenced the reaction

3.2.1. 1. The effect of pH

To reach to the optimum condition (high sensitivity, fast reaction and low absorbance for the blank solution), a study of dopamine with diazotizing compound 4-amino antipyrine in neutral, acidic and basic media was made as we notice there is no azo dye is formed in the neutral and the acidic media and the best media for the coupling reaction is the basic media which pH=10.80 as Table 3 represent the effect of PH on coupling reaction.

Table 3. The effect of PH on coupling reaction.

Kind of Buffer	pH	λ Max. (nm)	Abs.
Na_2HPO_4	2.6	317	0.18
Na_2HPO_4	7.00	430	0.29
Na_2CO_3-$NaHCO_3$	9.3	366	0.328
Na_2CO_3-$NaHCO_3$	10.4	444	0.368
Phosphate Buffer	10.8	449	0.661
Hydroxide- Chloride Buffer	13.8	433	0.285

5mL of buffer solution was used for every 25 mL of the final solution, the Table 3 shows that the best buffer solution for coupling reaction is phosphate buffer.

3.2.2. Type of Buffer solution

The effect of the type of buffer solution on the absorption was realized after the optimum value for the pH was found to be 10.80. Table 4 represent the influence of the buffer type on the complex absorbance.

Table 4. Influence of the of the buffer type.

Kind of Buffer	pH	λ Max.	Abs.
Na_2 HPO_4- NaOH	10.8	449nm	0.661
KCL- NaOH	10.8	449nm	0.357

3.2.3. The effect of the buffer volume on the intensity of absorbance

After fixing the optimum condition for the reaction. An experiment was made to find the optimum volume for buffer solution which gave the highest sensitivity Table 5 shows that 4mL represented the optimum volume.

Table 5. The volume of the buffer solution.

Volume of the Buffer solution (mL)	Abs.
2	0.214
2	0.312
3	0.254
4	0.661
5	0.362
6	0.245

3.2.4. Measurements the amounts of diazotized compound

The effect of the diazoting solution volume used for the absorbance measuring was studied. Table 6 shows that 3 mL represent the optimum.

Table 6 .Effect of diazoting solution volume on the absorbance.

Size of diazotizing solution (mL)	Abs.
1	0.254
3	0.661
5	0.153
6	0.321

3.2.5. Priority addition

In order to select the priority of order addition, three styles were investigated (Table 7). It was found that style 1 has the highest sensitivity and therefore, style 1 was chosen.

Table 7. Effect of addition order on the absorbance.

Order number	Reaction component	Abs.
1	A+D+W	0.661
2	W+D+A	0.243
3	W+A+D	0.352

were: D is buffer solution, W=Dopamine, A=diazotizing agent.

As we consider 1 as number one because it a high sensitivity.

3.2.6. Color stability

An experiment was done in order to explain the stability the colour of the complex formed due to the reaction between the DA and 4-amino antipyrine compound in the presence of phosphate buffer as shown in Table 8 the stability time for the colour of the complex is 30 min.

Table 8. Effect of time on the stability of the complex.

Abs.	T (min.)
0.177	5
0.267	10
0.423	15
0.324	20
0.421	25
0.661	30
0.581	60
0.426	120
0.325	1440

3.2.7. Calibration curve assay

Measurement of the calibration curve to a series of volumetric flask (250 mL) added 2 mL of diazotizing agent solution, 4 mL of phosphate buffer solution then pour to it (2-12 mL) of (50 $\mu g.mL^{-1}$) Dopamine solution after that complete with distal water until reach the mark then we measure the absorbance against the blank solution at 449 nm after 1/2 hr. from reaching the final solution (250 mL) and the Figure 3 show the calibration curve.

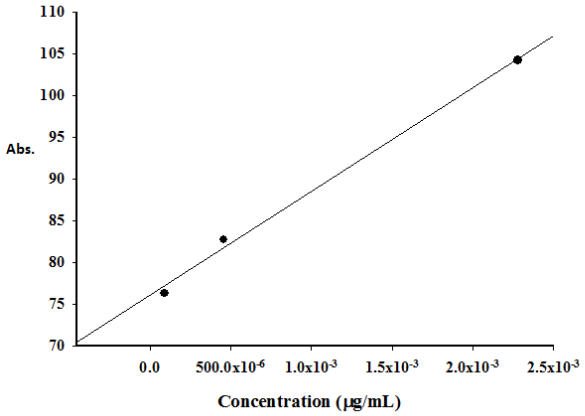

Fig. 3 Calibration curve of dopamine.

3.2.8. Study of accuracy and compatibility of method

A study for the accuracy and compatibility for the method was mad as we take a different concentration of dopamine (80, 25, 5) $\mu g.mL^{-1}$, by using an optimum condition, the Table 9 represent the result.

Table 9. Accuracy of different concentration of dopamine.

Represent value	Found value	Error %	Recovery %	R.S.D. %
5	10.55	0.56	96.7	±1.07
25	60.45	2.78	101.1	±1.23
80	98.45	0.89	99.3	±1.12

3.2.9. DA determination in blood sample

The characteristics of response recorded for the coupling reaction for micro determination of DA involved (linear concentration range of determination, selectivity, and sensitivity) revealed that this approached method can be used for the assessment of Dopamine in blood samples of persons taking this drug. The five samples of blood were supplied from the hospital, and used as collected, for the evaluation of (DA). Therefore, the results

obtained by using the coupling reaction between diazotized compound of 4- amino antipyrine and our target material (Dopamine) show that it is a reliable tool for the evaluation of (DA) in whole blood samples and Table 10 illustrate the result obtained.

Table 10. Recovery tests of P53 in blood samples.

Recovery of DA					
Samples Nr.	1	2	3	4	5
	5.65±0.07	6.34±0.08	4.48±0.09	4.78±0.08	5.12±0.07

4. CONCLUSIONS

The assay of Dopamine in whole blood was performed using the proposed coupling reaction based on coupling of diazotized 4-amino antipyrine with DA. The proposed method showed very good results for the recovery test which makes it a reliable tool for measuring dopamine measuring in whole blood samples, and this is very important for evaluation of trace amounts from DA.

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