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A COMPREHENSIVE REVIEW ON ESTIMATION OF BIOLOGICALAMINES BY USING UV SPECTROPHOTOMETRY

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ABSTRACT

Background: Biological amines are low molecular weight organic nitrogen compounds. A valid and accurate technique for quantitative neurotransmitter level measurement was developed. According to previous studies we report a simple and quick method for measuring the levels of dopamine (DA), nor epinephrine (NE), and serotonin (5-HT) in small brain tissue samples. The approach is based on brominating dopamine using an excess brominating mixture solution. Spectrophotometry was used to determine dopamine hydrochloride using potassium ferricyanide-Fe (III). The recommended approach is based on dopamine's inhibitory action on thionine oxidation by bromate in acidic environments. Objective: estimation of biologic amines by using UV Spectrophotometer. Conclusion: according to the previous studies by using UV Spectrophotometry the biologic amines estimation can be done. The suggested approach yielded good results in the determination of total serotonin derivatives in a strain of safflower seed extract, and is thus recommended as a regular method for total serotonin derivatives quantization. Dopamine hydrochloride was successfully tested in pharmaceutical, banana, urine, and serum samples. The sensitivity's reliance on the response variables was researched and improved in order to achieve maximum sensitivity. Under ideal experimental circumstances, the calibration curve was linear across the dopamine concentration range.

KEY WORDS: Biological amine, dopamine, serotonin, nor epinephrine UV Spectrophotometry.

INTRODUCTION

Neurotransmitters are one of the crucial for its role in attuning and adjusting of the central neural, hormonal, renal and cardiovascular system. Specific quantitative analysis of dopamine on tangled biological specimen quite crucial for a huge understanding of brain and its activity, as well as in the diagnosis, predicting and therapy of some ailments, such as Parkinson's disease, Alzheimer's disease and schizophrenia. So far many techniques have been progressed for the estimation of neurotransmitters. The current decade neurochemical measures have led to so much developments in understanding the correlation between CNS and behavioral, analytic, response state of an organism. Neurotransmitters content and release are also can describe by in vitro through analysis of cell in culture and ex vivo tissue composition such as brain slices. Many practical procedures having been implemented for the examination of the brain monoamines like fluorometric observation, chemiluminiscence observation, gas chromatography, capillary zone electrophoresis, liquid chromatography with mass spectroscopy, HPLC and etc. changes in the activity of adrenergic nervous system have been involved in many nourishment, physical and toxicological effects[1].

Fig1. Estimation of biologic amines by using UV Spectrophotometer

This region of brain is located deep in the brain stem where the dopamine produced in this area is responsible for neural communication between the striatum and the substantia nigra[2]. Parkinsonian symptoms appear when dopaminergic neuronal death exceeds a critical threshold of 70–80%. The decreased level of dopamine is directly associated with the uncontrolled motor function, which leads to the inability to neutralize the imbalance in neurotransmitters. In particular, the motor function in the striatum is dependent on the balanced equilibrium between dopamine and acetylcholine. The disruption in the balance of these two neurotransmitters can bring about the progression of PD. It is also known that nor epinephrine dysfunction is a contributing factor in PD, and serotonin and gamma(γ)-aminobutyric acid (GABA) may also affect the condition as secondary symptoms of PD. Furthermore, histamine, the initial neurotransmitter and immune mediator, has been reported to be significantly elevated in the brain with PD[3] . Monoamines (sometimes referred to as "biogenic amines") are three types of neurotransmitters as described in fig1.

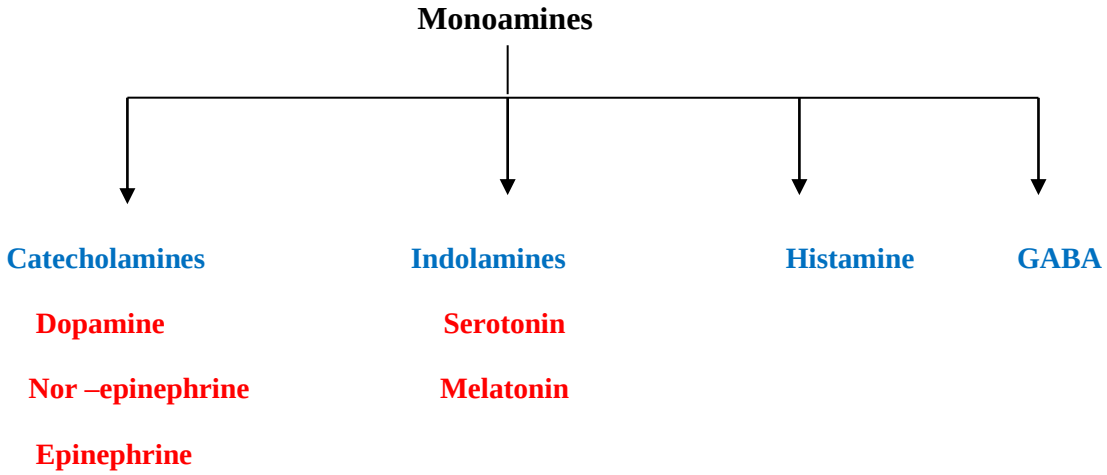
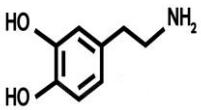
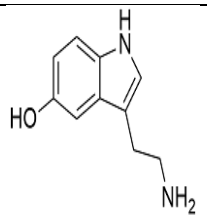
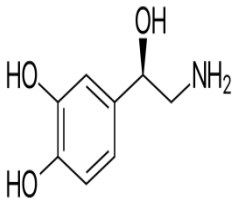
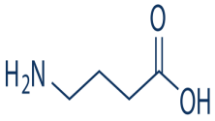


Fig2. Classification of biological amines

Dopamine (DA) is a key catecholamine neurotransmitter that is extensively distributed in the central nervous system. It is chemically known as 4-(2-aminoethyl)-benzene-1,2-diol. A variety of techniques have been used throughout the years to absorb the product at 735 nm. According to the DPH (Dopamine hydrochloride) determination. According to the previous studies Spectrophotometry was also employed to evaluate apparent molar absorption of coefficients of DPH determinations as $6.47 * 10^3$ and $7.4 *10^3$L mol^{-1} cm^{-1}, respectively. According to the previous studies spectrophotometry was a simple, sensitive, and low-cost test for DPH using potassium ferricyanide-Fe(III). Despite the fact that serotonin derivatives have several bioactivities, there are few easy yet reliable techniques for assaying this class of compounds, with the most often listed methods being high-performance liquid chromatography (HPLC). Although the HPLC method is suitable for determining the serotonin derivative in a structure-specific manner, it is time-consuming due to the demand for complex and difficult operation skills and multi-standard substances; additionally, in many cases the total rather than the structure-specific quantity is required, making the accuracy of the HPLC method unnecessary[7]. Additionally, preclinical and clinical research on GABAergic and glutamatergic pathways, as well as data on the effects of neuroactive steroids, indicate that GABA and glutamate play a significant role in the genesis of pain and depression, and may contribute to comorbidity[4]. GABA is a nonprotein amino acid that is generated by the e-decarboxylation of L-GIu in a process catalysed by glutamate decarboxylase. A technique of determining GABA is described that removes both GABA concentration after tissue sample and interfering pigments[5]. According to the previous studies the spectrophotometric techniques presented are based on the interaction of two catecholamines with sodium periodate in an aqueous alcoholic solution for estimation of nor norepinephrine[6].

Table1: Raised level of neurotransmitter and decreased level of neurotransmitter

Neurotransmitters	Structure	Mechanism of action	Raised level of neurotransmitter	Decreased level of neurotransmitter
1. Dopamine	 dopamine	dopamine produces positive chronotropic and ionotropic effects on the myocardium, resulting in increased heart rate and cardiac contractility[1].	more competitive, aggressive and having poor impulse control[1]	it's linked to some mental illnesses including depression, schizophrenia and psychosis[1].
2.Serotonin	 Serotonin	serotonin can also bind to auto-receptors on the presynaptic neuron to regulate the synthesis and release of serotonin[1]	too much serotonin causes signs and symptoms that can range from mild (shivering and diarrhea) to severe (muscle rigidity, fever and seizures)[1]	low levels of serotonin in the brain causes depression, anxiety, and sleep trouble[1]
3.Nor epinephrine		Nor adrenaline is a vasoconstrictor t hat predominantly stimulates α_1 receptors to cause peripheral vasoconstriction and increase blood pressure. It also has some	high blood pressure, anxiety, excessive sweating[1].	anxiety, depression , changes in blood pressure[1]

		β_1 receptor agonist activity that results in a positive ionotropic effect on the heart at higher doses[1].		
4.GABA		Gamma-amino butyric acid (GABA) is an amino acid that functions as the primary inhibitory neurotransmitter for the central nervous system (CNS). It functions to reduce neuronal excitability by inhibiting nerve transmission[4]	GABA blocks certain nerve signals in the brain to reduce fear, anxiety, and stress. Without the right level of GABA in the body, conditions such as anxiety disorders may become worse[4]	GABA helps to control fear and anxiety when neurons become overexcited. Lower-than-normal levels of GABA in the brain have been linked to schizophrenia, depression, anxiety, and sleep disorders[4]

Spectrophotometric method for determination of dopamine by bromination

A novel spectrophotometric method was designed for estimation of dopamine. The frame work was focused on brominating dopamine using an excess brominating mixture solution. Bromine was released from the brominating mixture at this point. In the previous studies the goal of research work was to provide a easy, quick, accurate, and repeatable spectrophotometric technique for estimating dopamine in pharmaceutical formulations. In a strong acidic medium, dopamine was brominated with the brominating mixture. Following bromination, the surplus brominating mixture was treated with potassium iodide to produce a yellow potassium triiodide complex with the highest color sensitivity and stability. The effects of the brominating mixture, hydrochloric acid, potassium iodide, and the order of additions were studied to improve the experimental conditions. The recovery assays were carried out by mixing several pure medicines into the examined tablet sample solution; the results are shown in the table. The recovery results vary from 98.6 to

100.2 percent, confirming that the procedure is accurate[7].According to the previous studies the by comparing the findings of the suggested and documented methods for each dose type, the method's accuracy was determined.

Spectrophotometric method for determination of dopamine by Potassium Ferric cyanide – Fe (III)

According to the previous studies by using potassium ferricyanide-Fe (III), we devised a simple, precise, and low-cost technique to measure dopamine hydrochloride by UV Spectrophotometry. The study reveals that at pH 4.0, dopamine hydrochloride deoxidizes Fe (III) to Fe(II), and subsequently Fe(II) interacts with potassium ferricyanide to generate a soluble Prussian blue (KFeIII [FeII (CN)6]). A spectrophotometer was used to track the absorbance of this product over time at an absorbance peak of 735 nm, and the concentration of dopamine hydrochloride may be estimated based on the absorbance. The concentration of dopamine hydrochloride vs absorbance had a satisfactory linear relationship, and the linear regression equation A = 0.022 + 0.16921C (ug mL–1) was generated. For the indirect measurement of dopamine hydrochloride, the apparent molar absorption coefficient was 3.2 104 L mol–1 cm–1. In the previous studies the described method was used to estimate the dopamine hydrochloride was successfully tested in pharmaceutical, banana, urine, and serum samples. DPH recovery rates varied from 98.6 to 101.8 percent in pharmaceutical, banana, urine, and serum samples, indicating that the common components in these samples had no effect on DPH assessment using this approach[8].

A novel spectrophotometric method for determination of dopamine in biological and Pharmaceutical samples

According to the previous studies the quantitative determination of dopamine was carried out by using sensitive and accurate technique. The suggested approach is based on the fact that dopamine inhibits thionine oxidation by bromate in acidic conditions. At 601 nm, the change in absorbance was measured spectrophotometrically. The relationship between sensitivity and reaction factors was explored and adjusted in order to get the highest degree of sensitivity. The calibration curve was linear across the range of 0.2–103.3µgmL1 of dopamine under ideal experimental circumstances. Some relative standard deviations were observed such as (n=6) of0.5, 1.0, 5.0, and 30.0 μgmL^{-1} of dopamine were 1.13, 1.02, 0.99, and 0.97% respectively. The limit of quantification was 0.057μgmL^{-1} of dopamine. Because of its metachromic qualities, thionine (also known as Lauth's violet), a dark green crys- talline powder thiazine base, is used as a basic stain in histology for mucin and chromatin. The oxidation of thionine by powerful oxidising agents produced a colourless substance. It was utilized in hydrazine, vanadium and ruthenium kinetic spectrophotometric experiments. The objective of this research is to develop a novel approach for quantifying dopamine. The approach is based on dopamine's inhibitory action on the oxidation of thionine by bromate in acidic conditions[9].

- In acidic environments, thionine in reduced form is oxidised by bromate, resulting in the generation of bromide and thionine in reduced form as a colourless product.

$$\textbf{Thionine (Red)} + BrO3- + H+ \rightarrow \textbf{Thionine (Ox)} + Br- \quad (1)$$

- A well-known interaction between bromide and bromate in acidic environments (reaction) reduces bromine production.

$$BrO3- + H+ + Br- \rightarrow Br2 + H2O \quad (2)$$

- Thionine oxidation is slowed by produced bromine, which is quicker than bromate oxidation.

$$\textbf{Thionine (Red)} + Br2 + H+ \rightarrow \textbf{Thionine (Ox)} + Br- \quad (3)$$

- A reducing agent (in this example, dopamine) interacts quickly with bromine, inhibiting the decolorizing reaction of thionine (reaction)

$$\textbf{Dopamine (Red)} + Br2 + H+ \rightarrow Br- + \textbf{Dopamine (Ox)} \quad (4)$$

- Because the level of inhibition is determined by the quantity of dopamine, establishing an analytical method for determining it is achievable[9].

Determination of serotonin by using UV Spectrophotometer (Ehrlich's reagent method)

In previous studies authors offer a novel spectrophotometric approach for determining total serotonin derivatives in safflower (Carthamus tinctorius L.) seeds. A colour reaction between serotonin derivatives and p-dimethylaminobenzaldehyde (Ehrlich's reagent) is used to make the determination, which follows the electrophilic substitution reaction mechanism at the indole ring. The fundamental parameters influencing the proper measurement of total serotonin derivatives concentration were examined. The complex's maximum absorption wavelength was determined to be 625 nm. Lambert-law Beer's is observed in the concentration range of 0.025–0.5 mmol/l, with a correlation coefficient (R2) of 0.9996, a recovery of 99.7 percent, and a relative standard deviation (RSD) of 1.5 percent. The suggested approach yielded good results in the determination of total serotonin derivatives in a strain of safflower seed extract, and is thus recommended as a regular method for total serotonin derivatives quantitation[10]The colour reaction, which is based on the creation of a violet, water-soluble complex of indole rings with p-dimethylami- nobenzaldehyde in accordance with the electrophilic substitution reaction mechanism, was successfully used to test total serotonin derivatives in safflower seeds. In compared to previous HPLC analytical techniques, the spectrophotometric methodology developed in this study is distinguished by its simplicity, specificity, adequate sensitivity, and low instrumentation costs. Further testing with different types of samples may prove that this approach is a viable option for regular measurement of total serotonin derivatives[7].

Determination of GABA by using UV Spectrophotometer

The isolation and testing of 7-aminobutyric acid (GABA) is given in detail. The extraction method prevents fast GABA buildup during tissue collection for examination. It also eliminates more than 95 percent of pigments that absorb at 340 nm, reducing the accuracy of the spectrophotometric associated enzyme test[5].

GABA + α – Ketoglutarate ⟶ glutarate + succinic aldehyde

Succinic semialdehyde + NADP ⟶ succinate + NADP

According to previous studies procedure for the estimation of GABA was designed.550µl sample containing 10-100nm GABA was taken.150 µl 4mM $NADP^+$ 200 µl 0.5MK^+ pyrophosphate buffer(P) 50 µl of 2 units GABAse per ml and 50 µl of 20mM of ketoglutarate was added. The initial absorbance was studied at 340nm before adding α-ketoglutarate, and the final absorbance was read after 10minutes.The commercial GABAse enzyme preparation was dissolved in 0.1M pyrophosphate buffer (P^H 7.2) containing 12% glycerol and 5mM 2- mercaptoethanol[5]

Determination of nor-epinephrine by using UV Spectrophotometer

According to the previous studies accurate and reliable spectrophotometric procedure for determining norepinephrine was presented. The approach was based on the formation of a red colour (λ_{max} 490 nm) in an aqueous alcoholic media with sodium periodate. The colour will remain constant for at least 1 hour. EP or NE has a molar reaction ratio of 1: 2 with periodate. The suggested approach was particularly well suited for regular NE injection analysis[6].

1ml of nor-epinephrine in 10ml of standard flask was taken

↓

1ml of acetone was added to the above step, and kept for 10min at room temperature

↓

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Add 2ml of sodium periodate solution and mix it

↓

Heat it for 2min at 60°C and cool it, up to room temperature, dilute the volume with ethanol

↓

Measure the absorbance at 450nm to 550nm[6]

CONCLUSION

The range of applications for UV–VIS spectroscopy is rapidly growing, with new uses being reported on a regular basis. Its popularity is due to its simplicity, precision, and cost efficiency in sectors like as pharmaceuticals, life sciences, foods, environmental research, forensics, and minerals. In today's world, it's impossible to conceive a laboratory without a UV-visible spectrophotometer. Biological amines can also be detected by UV–VIS spectroscopy. Derivative spectrophotometry is a useful analytical method for retrieving both qualitative and quantitative data from spectra with unresolved bands. In the present review different UV Spectrophotometric methods have been demonstrated for future use to estimate the biological amine.

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