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Colorimetric Studies for the Detection of Dopamine using Vanillin

H. R. KANAKASHREE, T. N. KISHORA and T. N. RAMESH*

Department of Studies and Research in Chemistry, University College of Science,
Tumkur University, Tumkur-572103, India.
*Corresponding author E-mail: adityaramesh77@yahoo.com

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ABSTRACT

Complex formation between dopamine hydrochloride and vanillin using a colorimetric method was investigated to develop a simple and effective approach for the detection and quantification of dopamine. Dopamine, a neurotransmitter with significant biological importance, reacts with vanillin, an aromatic aldehyde in ethanol, resulting in a color change (colorless to light brown) that was measured using colorimetry. The optimal conditions for complex formation with a specific molar ratio of dopamine hydrochloride to vanillin have been studied to establish a calibration curve correlating absorbance with dopamine hydrochloride concentration, allowing for the accurate quantification of dopamine hydrochloride. This colorimetric method provides a cost-effective, accessible, and reliable means of detecting dopamine, with potential applications in clinical diagnostics, pharmaceutical analysis, and neurological research. The above method offers a quantitative way to determine up to 4 ppm levels of dopamine hydrochloride.

Keywords: Colorimetric method, Vanillin, Dopamine hydrochloride, Color change.

INTRODUCTION

Dopamine is a critical neurotransmitter involved in various physiological processes, including motor control, mood regulation, and reward pathways[1]. Dopamine hydrochloride is a salt form of dopamine that has been shown to have a number of physiological effects and is used in the treatment of various conditions[2]. Accurate measurement of dopamine levels is essential in both clinical diagnostics and research, particularly for conditions such as Parkinson's disease, schizophrenia, and addiction, where dopamine dysregulation plays a significant role[3]. High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS) and Tandem Mass Spectrometry (MS/MS) methods provide high sensitivity and selectivity for the detection of dopamine hydrochloride[4-6]. Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS) have been used to identify the presence of the compound[7-9]. Other detection methods such as UV-Vis spectrophotometry, fluorescence spectrophotometry and electrochemical detection have been used to detect the presence of dopamine hydrochloride[10-12]. There are several challenges and limitations which we come across while using the above instrumentation/analytical techniques[13].

Dopamine hydrochloride can be affected by interference from matrix components, such as proteins, lipids and other substances present in biological samples[14,15]. Also, the detection methods mentioned above have limited sensitivity, which makes it difficult to detect low levels of dopamine hydrochloride[16]. Sample preparation is critical for accurate analysis and improper preparation can lead to false results or incomplete detection. Also the traditional methods are often expensive, time-consuming, and require sophisticated equipment and technical expertise. In contrast, chromogenic reactions involve the formation of a colored product, which can be used in the active pharmaceutical industry. Dopamine has been used as a chromogenic agent in analytical chemistry, particularly in the detection of certain important compounds[17-19]. Dopamine hydrochloride reacts with catecholamines, such as norepinephrine and epinephrine, which are important neurotransmitters[20]. Dopamine hydrochloride also reacts with (i) copper(II) salts to form a blue-colored complex, (ii) cobalt (II) salts to form pink coloured complex and iii) iron(III) salts to form red colour complexes, which are often used as an indicator for the detection of catecholamines[21-23]. Dopamine hydrochloride has been used as a reagent for the fluorescence detection of catecholamines, using fluorescence spectrophotometry[24]. Thus, dopamine hydrochloride offers several advantages as a chromogenic agent, including high sensitivity, selectivity and ease of use. Vanillin is a well-known flavoring and fragrance compound used to detect and quantify the presence of sugars (glucose, fructose), amino acids (tryptophan and tyrosine), and other analytes[25-28]. The bioactive properties of vanillin, such as neuroprotection, anticarcinogenic and also others have been well established. Vanillin offers several advantages as a chromogenic agent, due to high sensitivity and specificity, ease of use, low cost, wide range of applicability[29]. The reaction conditions for vanillin's utility as a chromogenic agent is dependent on the specific application and analyte being detected. Typically, the reaction involves mixing the analyte with vanillin in a suitable solvent, followed by incubation at a specific temperature and pH.

Dopamine and vanillin may not have direct interaction, while in a biological system the two act complementary to each other. Research has shown that dopamine is involved in the processing of sweet tastes, and vanillin is a key component of this process. On interaction of vanillin with L-DOPA, it gets converted to dopamine, thus dopamine release is triggered by the activation of sweet taste receptors[36]. This suggests that the interaction between dopamine and vanillin plays a role in the processing associated with eating sweet foods and influences food preference in humans and animals mediated by the activation of dopamine receptors[30,31]. The interaction between dopamine and vanillin is complex and multifaceted, involving sensory processing, reward processing, neuroplasticity and psychological effects[32]. Vanillin molecules bind to dopamine receptors in the brain through ligand-receptor binding where vanillin molecules interact to switch specific binding sites on the dopamine receptor, triggering a cascade of molecular events ultimately influencing mood and behavior. Vanillin has been found to bind to both D1-like and D2-like dopamine receptors, which are involved in the processing of reward and pleasure[33].

The oxygen molecules present in vanillin and dopamine molecules favor hydrogen bonding formation between the vanillin molecule and the dopamine receptor[34,35]. If large excess of unreacted vanillin is present, then the question arises whether there could be any interaction ? The colorimetric method offers a simpler, cost-effective alternative for dopamine detection[36,37]. Depending on the color change that occurs when dopamine reacts with specific reagents, such as vanillin[37]. Vanillin forms a colored complex with dopamine through a condensation reaction, which can be easily monitored by measuring the intensity of the color produced. The formation of the dopamine-vanillin complex provides a convenient basis for developing a colorimetric assay to detect and quantify dopamine. This method has several advantages, including simplicity, low cost making it suitable for use in clinical laboratories. Furthermore, the ability to visually observe the color change enhances the method's practicality, particularly where access to advanced instrumentation is limited. Till date, there are no reports to develop and optimize a colorimetric method for the detection of dopamine by investigating the complex formation between dopamine and vanillin. We have systematically explored the effect of different reaction conditions, such as concentration and reaction time on the colorimetric response during the interaction of dopamine hydrochloride with vanillin. In this article, dopamine hydrochloride

and vanillin are prepared at different concentrations and on mixing it in different mole ratios, the formation of dopamine hydrochloride-vanillin complex was investigated using colorimetry.

MATERIALS AND METHODS

Reagents used

Ethanol, procured from commercial source. dopamine hydrochloride, procured from Sisco Research Laboratories Private Limited, India. Vanillin, procured from SD Fine Chemicals Private Limited, India and used without purification.

Preparation of solutions

Dopamine hydrochloride (0.02 M): 0.075 g of dopamine hydrochloride was dissolved in 20 mL of ethanol.

Vanillin (0.02 M): 0.060 g of vanillin was dissolved in 20 mL of ethanol.

Protocol

(i). The cleaned and labeled test tubes from 1 to 6 are taken.

(ii). 5, 4, 3, 2, 1 mL of 0.02 M of dopamine hydrochloride was transferred into corresponding test tubes, respectively (from 1 to 5).

(iii). 1, 2, 3, 4, 5 mL of 0.02 M vanillin was added to the above test tubes separately.

(iv). Test tube No. 6 is treated as blank, which is filled with 6 mL of ethanol.

(v). The above solution mixtures were thoroughly stirred and allowed to equilibrate for 4 h 10 minutes.

(vi). The pale brown color was developed. Absorbance was recorded at different wavelengths with the solution mixture having maximum intensity (5th test tube-mixture) at different wavelengths and the λ_{max} was observed at 450 nm.

(vii). Corresponding to this λ_{max}, the absorbance of the above solution mixtures (in test tubes 1-4) was recorded.

(viii). Also, the absorbance values were recorded for the dopamine hydrochloride taken in different concentrations such as 0.02 M, 0.01 M, 0.005 M, 0.0025 M, 0.00125 M, 0.000625 M with a fixed concentration of vanillin (0.02 M).

(ix). The graph is plotted by taking absorbance versus concentration.

In Table 1 is given the detailed protocol containing dopamine hydrochloride and vanillin and their absorbance values recorded from the experimental data.

Table 1: Protocol to determine the mole ratio of dopamine hydrochloride and vanillin in a solution mixture using colorimetry

Test tube	Dopamine hydrochloride(0.02 M) (mL)	Vanillin (0.02 M) (mL)	Absorbance (450 nm)
1	5	1	0.09
2	4	2	0.11
3	3	3	0.14
4	2	4	0.35
5	1	5	0.37
6	Blank	-	0.00

Since maximum intensity was observed for test tube 5 containing dopamine hydrochloride (1 mL of 0.000625 M) and vanillin (5 mL of 0.02 M vanillin). The concentration of vanillin (0.02 M) is fixed and the dopamine hydrochloride concentration was varied. The above mixtures are allowed for 6 h 30 min to equilibrate and absorbance values were recorded. After equilibrating the above mixtures for 22 h 15 min, the absorbance values were re-recorded.

Characterization

An Elico CL-63 colorimeter was used for the absorbance measurements. Different filters are used to measure absorbance maxima in the range of 400-700 nm.

RESULTS AND DISCUSSION

The structure of dopamine hydrochloride and vanillin are shown below [see Figures 1(a) and 1(b)][35,37].

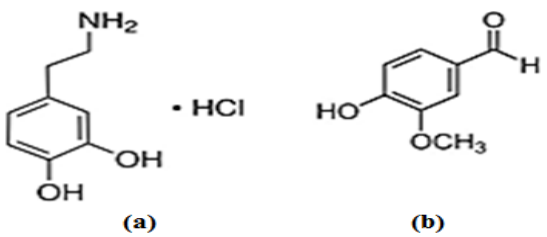

Fig. 1. Molecular structures of dopamine ($C_8H_{11}NO_2$.HCl) and vanillin ($C_8H_8O_3$)

In Fig. 2(a) the absorbance maxima for the solution mixture containing dopamine hydrochloride and vanillin are shown. The maximum wavelength at highest absorbance was recorded at 450 nm. For all the subsequent measurements, the wavelength of the filter was set at 450 nm in the colorimetry. In Fig. 2(b) shows the variation in the absorbance at different dopamine hydrochloride/vanillin mole ratios. The data indicates that dopamine hydrochloride to vanillin at the lowest mole ratio had maximum absorbance. The concentration of dopamine hydrochloride (0.02 M; 1 mL) and vanillin (0.02 M; 5 mL).

In Fig. 2(c) is shown the variation in the absorbance with an increase in the concentration of dopamine hydrochloride at a fixed concentration of vanillin (0.02 M) after 6 h 30 minutes. The absorbance values were re-recorded for the above samples after 22 h 15 min and the data is shown in Figure 2(d).

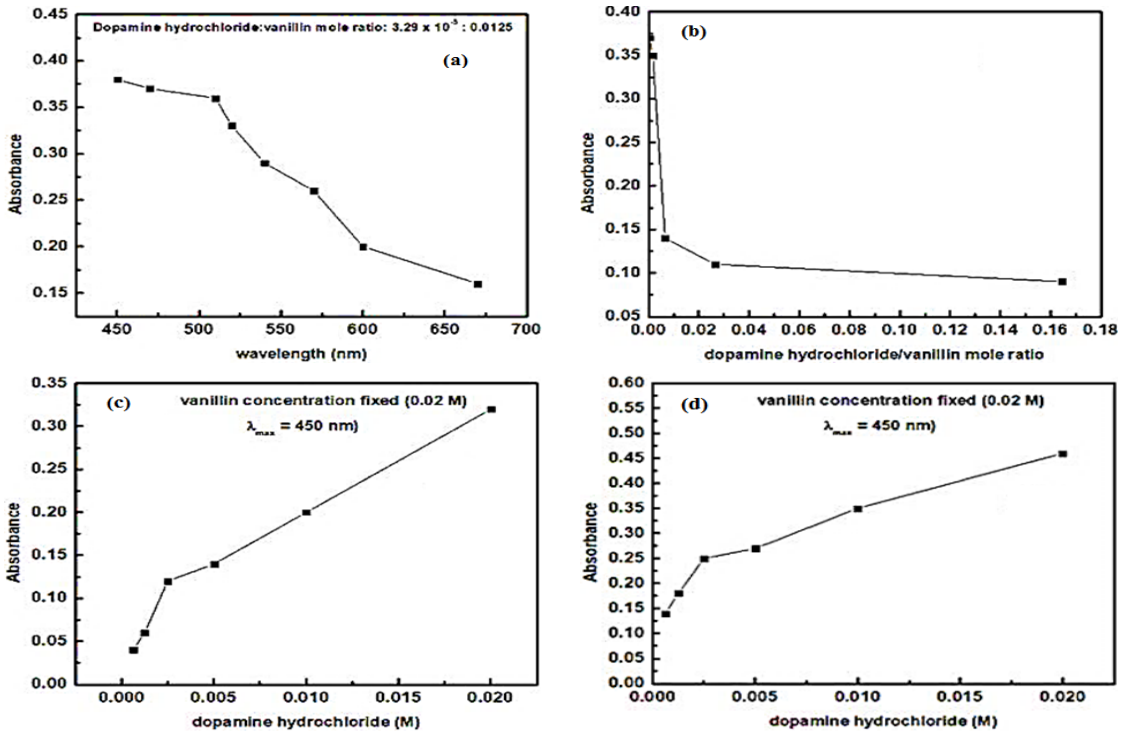

Fig. 2(a). Absorbance maxima (λ_{max}) at a fixed mole ratio of dopamine hydrochloride/vanillin; Fig. 2(b). Absorbance v/s mole ratio of dopamine hydrochloride v/s vanillin; Fig. 2(c). Dopamine hydrochloride concentration variation at a fixed concentration of vanillin (0.02 M) after 6 h 30 minutes; Fig. 2(d). Dopamine hydrochloride concentration variation at a fixed concentration of vanillin (0.02 M) after 22 h 15 minutes

From the data it is clearly evident that the stability of the dopamine-vanillin complex increases at longer duration, which was reflected in the increase in the intensity of the solution mixture with time.

Thus, there could be the formation of a dopamine-vanillin complex which is soluble in ethanol and quite stable, resulting in the formation of a Schiff base/conjugated system[38,39]. We could detect up to 4 ppm of dopamine in dopamine hydrochloride using vanillin as a chromogenic agent and there are no reports till date using the above method.

CONCLUSION

The colorimetric analysis of the reaction between dopamine hydrochloride allows for the quantitative measurement of dopamine concentration. When dopamine hydrochloride reacts with vanillin, a specific color change occurs, indicating the formation of a complex. This color change is typically the result of the formation of a Schiff base or another type of conjugated system, which absorbs light at 450 nm. This experiment demonstrates the formation of a colored complex between dopamine hydrochloride and vanillin, which

can be quantified through colorimetric analysis up to 4 ppm. This technique also provides insight into the chemical interactions between dopamine hydrochloride and vanillin and is valuable for both qualitative and quantitative assessment of dopamine.

Declaration

All authors declare no conflict interestinterest, including any financial, personal or other relationships with other people or organizations that can influence the work.

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