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A New Spectrophotometric Determination of Adrenaline with CDTA

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Adrenaline is an hormone secreted by the medulla part of the adrenal gland. Adrenaline is estimated by a new and direct spectrophotometric method after developing a colour with ammonium molybdate, sodium nitrite in presence of cyclohexylene 1,2-dinitrilotetraacetic acid (CDTA) in aqueous medium. Various experimental parameters that affect the colour saturation, like pH, time stability of the colour developed, concentration of reactants, order of addition, were studied and optimised. Interference due to many foreign ions in the estimation of adrenaline was also studied and tolerance limits were determined.

INTRODUCTION

A number of methods were reported to find application in the determination of adrenaline (catecholamine). Loew[1] observed that alkaline oxidation of adrenaline yielded a yellow green fluorescent product. This reaction was explored further by Gaddum and Schild[2]. Lund described the first fluorimetric method[3] for the assay of adrenaline and noradrenaline in plasma based on the difference in oxidation rate. Notelson[4] examined the reaction between adrenaline, ammonia and a number of organic primary amines and was the first to describe an assay for adrenaline using EDA, which was then applied to the analysis of plasma by Weil-Malharbe and Bone[5]. However, a differential assay for adrenaline and nor-adrenaline was described[6] which was based on the difference in their respective fluorescence emission spectra. In 1932 Barker et al.[7] reviewed the many colorimetric assays for the determination of adrenaline and other catecholamines. Bloor and Bullen[8] evaluated a number of colorimetric assays and attempted to estimate human venous plasma adrenaline concentration, but found all methods lacked reproducibility.

In the present study we report here on the spectral behaviour of adrenaline with ammonium molybdate, sodium nitrite and CDTA in aqueous medium. After establishing the optimum conditions for the colour saturation, the same were utilized for the spectrophotometric determination of adrenaline.

EXPERIMENTAL

Materials and Methods

A Beckman spectrophotometer (Model 26, U.S.A.) with quartz cell of 1 cm path length was used for the spectral studies and spectrocolorimeter

(Systronics, Model 103, India) was used for absorbance measurements. pH of the solution was adjusted with the help of a digital pH-meter (Naina, NIG 333, India) fitted with glass electrodes.

Adrenaline (Sigma Chemical Company, U.S.A.) solution was prepared to give 0.001 M solution with addition of 0.5 ml of conc. sulphuric acid. The reagent ammonium molybdate (Sarabhai M. Chemicals, India) 0.001 M and sodium nitrite (Sarabhai M. Chemicals, India) 0.001 M solutions were prepared in distilled water. A 0.001 M solution of CDTA (E. Merck, Germany) was prepared adding a few drops of sodium hydroxide solution in distilled water to get clear solution. All the solutions used were prepared from A.R. grade reagents.

Preliminary investigations were carried out to find the optimum pH range for the estimation; it was observed that the absorption maximum at λ 475 nm persists (when using a mixture of adrenaline with ammonium molybdate, sodium nitrite and CDTA) over a range of pH from 0.5 to 4.5, the optimum being 3.2 to 4.0, where the absorbance value remained constant. Hence pH 3.7 ± 0.1 was chosen for further studies as colour saturation.

The effect of concentration variation of ammonium molybdate sodium nitrite or CDTA on colour saturation were studied in detail.

Variation of Ammonium Molybdate Concentration

Adrenaline 8.0×10^{-5} M, sodium nitrate 4.0×10^{-5} M, CDTA 4.0×10^{-5} M and varying concentration of ammonium molybdate in the range 0.2×10^{-5} M–3.2×10^{-5} M were mixed at pH 3.7 ± 0.1 and absorbance was measured at λ 475 nm. The data indicates that colour saturation for this system was obtained in the concentration range 2.0×10^{-5} M–3.2×10^{-5} M of ammonium molybdate. Hence the concentration of 2.4×10^{-5} M of this reactant was chosen for further studies.

Variation of Sodium Nitrite Concentration

The effect of sodium nitrite concentration on colour formation indicate that colour intensity increases with increasing concentration of sodium nitrite solution to a mixture containing 3.0×10^{-5} M adrenaline, 2.4×10^{-5} M ammonium molybdate and 4.0×10^{-5} M CDTA solution at pH 3.7 ± 0.1. The absorbance value was taken at λ 475 nm. The data indicate that colour saturation was obtained at 6.0×10^{-5} M to 8.0×10^{-5} M of sodium nitrite concentration range. Hence the concentration of 7.2×10^{-5} M of this reactant was chosen for further studies.

Variation of CDTA Concentration

For the above system adrenaline 8.0×10^{-5} M, ammonium molybdate 2.4×10^{-5} M, sodium nitrite 7.2×10^{-5} M and varying concentration of CDTA in the range 0.2×10^{-5} M–1.4×10^{-5} M were mixed at pH 3.7 ± 0.1

and absorbance was measured at λ 475 nm. The data indicate that colour saturation for this system was obtained in the range 1.0×10^{-5} M–1.4×10^{-5} M of CDTA. Hence the concentration 1.2×10^{-5} M of this reactant was chosen for further studies.

Time Effect

The absorbance value of the mixture containing 8.0×10^{-5} M adrenaline, 2.4×10^{-5} M amonium molybdate, 7.2×10^{-5} M sodium nitrite and 1.2×10^{-5} M CDTA solution was measured at different time intervals at pH 3.7 ± 0.1, and absorbance values were measured at λ 475 nm. The data indicate that colour saturation for this system was obtained 55 minutes after mixing the solution and remains constant up to 120 minutes. Hence work was carried out after 60 minutes and before 120 minutes of preparation of solutions.

The optimum concentration range for the estimation of adrenaline was found to be 1.47–21.98 μg/ml and conforms to Beer's law. Sandell sensitivity index[9] for A = 0.001 and 1 cm path length was 0.052 μg/ml of adrenaline and molar extinction coefficient is 3500. The relative standard deviation for six samples at 14.71 μg/ml (8.0×10^{-5} M) level was 0.46% and confidence limit[10] at 99% C.L. is 14.63 ± 0.101.

Recommended Procedure

In aqueous medium 2.4×10^{-5} M amonium molybdate, 7.2×10^{-5} M sodium nitrite, 1.2×10^{-5} M CDTA and varying concentration of adrenaline solution in the range 8.0×10^{-6} M–1.4×10^{-4} M were mixed and pH was adjusted to 3.7 ± 0.1. The absorbance values were taken at λ 475 nm against reagent blank after 60 minutes of mixing the solution and before 120 minutes. The concentrations of the solutions given are those calculated after their dilution to a fixed volume (25 ml) with distilled water of the same pH in all these cases. Adrenaline was estimated from the standard Beer's law plot in an unknown solution prepared in a similar way.

Effect of Foreign Ions

A number of representative ions were examined for their interference in the determination of adrenaline by the recommended procedure. The following foreign ions (tolerance limits in parentheses) did not show significant interference in the estimation of 183.2 μg of adrenaline taken in a total volume of 25 ml.

Li^+ (16.5 μg),	Na^+ (35.0 μg),	K^+ (48.5 μg),
NH_4^+ (20.0 μg),	Ag^+ (1.0 μg),	Hg^{++} (0.3 μg),
Mn^{++} (31.0 μg),	Cu^{++} (37.5 μg)	Ni^{++} (40.5 μg),
Co^{++} (38.0 μg),	Ba^{++} (21.5 μg),	Sr^{++} (16.0 μg),

Ca^{++} (26.0 μg), Fe^{+++} (14.8 μg), Cl^{-} (16.5 μg),
ClO_3^{-} (8.0 μg), Br^{-} (12.0 μg), I^{-} (3.5 μg),
CH_3COO^{-} (15.0 μg), $C_2O_4^{--}$ (15.0 μg), SO_4^{--} (0.8 μg)
$C_4H_4O_6^{--}$ (6.0 μg).

The method deserves special mention of its selectivity of its selectivity in the sense that interference from foreign ions is much less than that in case of other methods for the determination of adrenaline.

RESULTS AND DISCUSSION

Adrenaline in presence of ammonium molybdate gives an intense yellow colour with absorption band in the range of 325–350 nm. However, in presence of CDTA the colour intensity of the system decreases. Adrenaline in presence of sodium nitrite gives a light pink colour with an absorption band in the range of 460–500 nm, but the colour fades with time. If sodium nitrite and CDTA are present together in the system of adrenaline and ammonium molybdate the orange colour intensifies and shows an absorbance maximum in the range of 470–485 nm. The colour intensity of the solution remains stable in this case.

To confirm that this colour was obtained only with this combination of reactants, various alternative combinations of mixtures, like ammonium molybdate + sodium nitrite, ammonium molybdate + CDTA, sodium nitrite + CDTA, ammonium molybdate + sodium nitrite + CDTA, adrenaline + CDTA (all these colourless), adrenaline + ammonium molybdate (yellow colour, λ_{max} 320–350 nm), adrenaline + sodium nitrite (light pink colour, λ_{max} 460–500 nm), adrenaline + ammonium molybdate + CDTA (light yellow colour, λ_{max} 330 nm), adrenaline + sodium nitrite + CDTA (light pink colour, λ_{max} 460–500 nm), adrenaline + ammonium molybdate + sodium nitrite (orange unstable colour, λ 465–495 nm), were prepared at the same pH and scanned their absorption bands are given in parentheses. This confirms that the combination of adrenaline, ammonium molybdate, sodium nitrite and CDTA will alone give a stable orange red colour with λ_{max} 475 nm. An additional advantage is the presence of CDTA which can eliminate many potential interferences.

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