Spectrophotometric determination of benzidine present in pure sample and in industrial effluents

M.A.Sathish, M.S.Yogendra Kumar, Mansour S. Abdul Galil, Mahadevaiah, B.Shyla, C.Vijaya Bhaskar, G.Nagendrappa*
Department of studies in Chemistry, University of Mysore, Manasagangotri, Mysore-570006, (INIDA)
E-mail: gnagendrappa@yahoo.co.in
Received: 8th December, 2008; Accepted: 13th December, 2008

ABSTRACT

Two simple, rapid, economical and highly sensitive spectrophotometric methods have been developed for the determination of benzidine (BZ) present in its pure samples and in industrial effluents. The methods are based on oxidation of benzidine either with cerium(IV)sulfate in sulfuric acid medium (method A) or with electrochemically generated manganese (III) (method B). The colored ion-pairs formed are measured at 430 and 410 nm for methods A and B respectively. Beer’s law is obeyed in the concentration range of 0.10-0.71 (method A) and 0-4 μg ml⁻¹ (method B). The respective molar absorptivities are 6.27×10⁴ and 3.52×10⁴ l mol⁻¹ cm⁻¹ with the corresponding Sandell sensitivities values 0.0029 and 0.005 μg cm². The stability constants values of the complexes as determined by modified Job’s method are 2.11×10⁵ and 4.23×10⁴ l mol⁻¹ respectively for methods A and B. The new methods have been applied successfully for the analysis of benzidine in pure form or in industrial effluents with good accuracy and precision. The results from the developed methods are in good agreement with the reported method. The mechanism of benzidine oxidation either with cerium(IV)sulfate or manganese(III) is also proposed. The stoichiometry between benzidine and cerium(IV)sulfate or manganese(III) is 1:2 for both the methods. © 2009 Trade Science Inc. - INDIA

INTRODUCTION

Benzidine,[1,1′-biphenyl]-4,4′-diamine, is classified as a carcinogen⁴⁻⁻⁴, found in the environment either in its free state (as an organic base), or as chloride or sulfate salts. It is a toxic chemical but used extensively in the synthesis of dyes⁵ and wide range of organic chemicals and also as an analytical reagent⁶⁻⁻⁸.

Several methods are available in the literature for its determination. The methods include, capillary zone electrophoresis⁹, supercritical fluid chromatography¹⁰, gaschromatography-mass spectrometry¹¹⁺¹², gaschromatography¹³, HPLC¹⁴⁻⁻²³, mass spectrometry/capillarygas chromatography²⁴, voltammetry²⁵ and spectrophotometry²⁶⁻⁻³⁰. A few spectrophotometric methods are also available for the determination of benzidine based on its diazotization followed by its coupling either with α-naphthol in acidic medium³¹ or with α-naphthol in presence of 8-hydroxy quinoline³² or with salicylic acid³³ or with 3-hydroxy-1, 2, 3, 4- tetrahydrobenzo(h) quinoline in aqueous ethanol solution³⁴ or by converting benzidine into nitrobenzene and using Fe(III) and 1,10 phenanthroline as color forming reagents³⁵. It is also determined by oxidation of benzidine either with perchlorates followed by extraction of chromophoric compound into chloroform³⁶ or with sodium perchlorate in sulphuric acid³⁷ medium. However, some of the exist-
ing methods are appearing to be suffering from one or the other disadvantages like, pretreatment of benzidine, requiring extraction, involve hazardous chemicals and some require expensive sophisticated instruments. Literature survey reveals that various oxidants have been used for the determination of benzidine. Though, cerium(IV) sulfate and electrolytically generated Mn(III) are oxidizing agents, it appears from the literature that they have not been used for spectrophotometric determination of benzidine. Therefore considering the importance of benzidine as an analytical reagent, hazardous carcinogen and also the limitations associated with the reported methods, an attempt has been made here to develop simple, rapid, economical and sensitive spectrophotometric methods for determination of benzidine either in pure form or in industrial effluents employing cerium(IV) sulfate and electrolytically generated Mn(III) as oxidants.

**EXPERIMENTAL**

**Apparatus and reagents**

UV-1700 Pharmaspec Spectrophotometer (Shimadzu), spectrophotometer Elico SL 171 mini spec (Hyderabad) with 1cm matched optical cells and Platinum Electrodes (Arora–Mathey, Kolkata) were used.

Benzidine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), cerium(IV) sulphate, sodium nitrite, manganese(II)sulphate, magnesium sulphate, potassium nitrate, sodium chloride, hydrochloric acid, sulphuric acid, p-nitroaniline and p-anisidine (Ranbaxy Fine Chemicals Ltd, New Delhi, India) were used. Millipore water was used for preparing the solutions.

**Preparation of cerium(IV) sulphate solution**

It was prepared by dissolving 0.01011g of cerium(IV) sulphate in 2M sulphuric acid and diluted to 250 ml with 2M sulphuric acid. The concentration of the prepared solution was determined by reported method and the solution was found to be 5×10⁻⁴M.

**Preparation of Mn(III) solution**

A known volume, 10 ml, 2M manganese(II) sulphate solution was diluted to about 100 ml with 5M sulfuric acid in a beaker. It was subjected to electrolytic oxidation using platinum electrodes for about 2hr. The rose red Mn(III) solution obtained was standard-ized by iodimetric method and the solution was found to be 0.0163M. From this solution, an aliquot of 6.1ml was further diluted to 100 ml with 5 M sulfuric acid to obtain 0.001M solution.

**Preparation of standard benzidine solution**

Accurately weighed amount of pure sample of benzidine (10.11mg) was taken in a beaker. It was dissolved in 5-10ml of ethanol and transferred it into a 100ml volumetric flask. The beaker was washed with water and washings were also transferred into the flask. Then the solution was diluted to the mark with water. From this solution; an aliquot of 10ml was further diluted to 100ml with water to obtain 5.54×10⁻⁵M solution.

**Procedure for determination of benzidine**

**Method A**

A known but various volumes, 0.1-0.7ml of 5.54×10⁻⁵M benzidine solution were transferred into a series of 10ml labeled volumetric flasks. To each flask, a measured volume, 0.1ml of 5×10⁻⁴M cerium(IV) sulphate solution was added. The contents of the flasks were mixed well and the solution in each flask was diluted to the mark with 2M sulphuric acid. After 5min, absorbance of each solution was measured at 430nm against the reagent blank which was prepared under identical conditions but without adding benzidine solution.

**Method B**

A known but various volumes, 0.1- 4 ml of 5.54×10⁻⁴M benzidine solution were transferred into series of 10 ml labeled volumetric flasks. To each flask, a measured volume, 0.5ml of 0.001M Mn(III) solution was added. The contents of the flasks were mixed well and the solution in each flask was diluted to the mark with water. After 15min, absorbance of each solution was measured at 410nm against the reagent blank which was prepared under identical conditions but without adding benzidine solution.

**Procedure for the assay of industrial effluents**

A known volume, 100ml of an industrial effluent, suspected to be containing benzidine was extracted with 100ml chloroform in two 50ml fractions. The combined extract was taken in a 250ml beaker and evaporated the contents to dryness on a water bath. The residue
obtained was dissolved in 5ml ethanol and transferred it into a 25ml volumetric flask. The beaker was washed with water and transferred washings into the flask. Then the solution was diluted to the mark with water. An aliquot of 1ml solution was analyzed by both the methods for its benzidine content following the recommended procedures.

RESULTS AND DISCUSSION

The reaction conditions as well as the various experimental parameters affecting the development and stability of the colored product formed in both the methods were carefully investigated and optimized for the quantitative determination of benzidine in pure form and in industrial effluents. For a comparison of the results, benzidine was also determined separately by following reported method [26] for industrial effluents. The results obtained form the developed methods are in good agreement with the reported method [26] involving diazotization, coupling with \( \alpha \)-naphthol in acidic medium, and extraction of the violet dye formed in alkaline solution into 3-methyl-1-butanol.

Method A

Effect of cerium (IV) sulphate

The effect of cerium(IV) on the color intensity of the chromophore at constant benzidine concentration (0.408 \( \mu \)gml\(^{-1}\)) was examined with various volumes (0.05 to 0.5ml) of 5 \( \times \) 10\(^{-4}\)M cerium(IV) sulphate solution. The absorbance of each solution and the blank (same as the test solution but without cerium(IV) sulphate) were measured against 2M sulphuric acid at 430nm. The results obtained are shown in (Figure 1) accounting for the maximum absorbance value with 0.1ml of 5 \( \times \) 10\(^{-4}\)M cerium (IV) sulphate solution but above that the absorbance of the solution was found to be decreasing. Therefore, 0.1 ml of 5 \( \times \) 10\(^{-4}\)M cerium (IV) sulphate solution was used in all further spectral measurements.

Effect of different diluents on color stability and intensity

In order to understand the effect of different diluents on color intensity of the solution containing benzidine and cerium(IV), 0.4ml benzidine solution was pipetted into each one of the six labeled 10ml volumetric flaks. To each flask, 0.1 ml of 5 \( \times \) 10\(^{-4}\)M cerium(IV)sulphate solution was added. Solution in each flask was diluted separately up to the mark with water, 0.5M sulphuric acid, 1M sulphuric acid, 2M sulphuric acid, 3 M sulphuric acid and 1M hydrochloric acid. The absorbance of each solution was measured at 430nm against the respective blank solution. The absorbance values obtained with different diluents are shown in TABLE 1 indicate that, the solutions, which were diluted with 0.5M sulphuric acid, 1M sulphuric acid, 3M sulphuric acid and 1M hydrochloric acid were found to have lower absorbance values than the solution that was diluted with 2M sulphuric acid. The solution diluted with water was having the same absorbance value as that of the solution diluted with 2M sulphuric acid. Further, the solution that was diluted with water was found to be decreasing its color very rapidly with time and finally discharged its color completely in about 20min. Therefore, 2M sulphuric acid was used as a diluent through out the experimental investigations.

Method B

Effect of different diluents on color intensity

In order to understand the effect of different diluents on color intensity of the solution containing benzidine and Mn(III),2.5ml benzidine solution was pipetted into each one of the seven labeled 10ml volumetric flaks. To each flask, 0.5 ml of Mn(III) sulfate solutions was
added. Solution in each flask was diluted separately up to the mark with water, 0.5M sulphuric acid, 1M sulphuric acid, 2M sulphuric acid, 3M sulphuric acid and 1M hydrochloric acid. The absorbance of each solution was measured at 410nm against the respective blank solution. The absorbance values obtained with different diluents are shown in TABLE 1 indicate that, the solutions, which were diluted with 0.5M sulphuric acid, 1M sulphuric acid, 2M sulphuric acid, 3M sulphuric acid and 1M hydrochloric acid were found to be having lower absorbance values than the solution that was diluted with water. Therefore, water was used as a diluent through out the experimental investigations.

Absorption spectra

The absorption spectra of benzidine chromophore obtained from the reaction of benzidine either with Ce(IV) or with Mn(III) shows maximum absorption at 430 and 410nm for method A and B respectively and their respective absorption spectra are shown in (Figures 2 and 3).

Effect of time on color

The color stability of the chromophore was studied according to the recommended procedures for 0.4ml of $5.54 \times 10^{-5}$M benzidine solution. The absorbances of the solutions were measured at 430 and 410nm respectively for method A and B at different intervals of time. The absorbance values indicate that, the system attained maximum color intensity in about 5 and 15min and are found to be stable for 30 and 25min respectively for method A and B. Afterwards, the solutions were found to be decreasing their absorbances slightly with time but retained their color for about 2hrs. Though the solutions prepared under specified experimental conditions had shown a time dependent absorbance, that were not affecting the results of the benzidine since the standard and the test solutions of benzidine were prepared almost at the same intervals of time.

Determination of stoichiometry and stability constant

The composition between benzidine and Ce(IV)(method A) and benzidine and Mn(III)(method B) were studied by modified Job’s method of continuous variation[48], the concentration of aqueous solutions of Ce(IV) or Mn(III) and benzidine were 0.001M. Nine, 10ml volumetric flasks containing Ce(IV) or Mn(III) and benzidine solutions in various molar ratios were prepared so that their volumes were always amounted to 1ml. Then, the solutions were diluted to the mark with 2M sulphuric acid for method A and with water for method B. The absorbances of the solutions were measured at 430 and 410nm respectively for method A and B. Similarly, repeated the experiment with same molar ratios of Ce(IV) or Mn(III) and benzidine but the solutions were diluted to 25ml. The results obtained were used in plotting the graphs (Figures 4 and 5), which indicate 1:2 stoichiometry between benzidine and Ce(IV) or Mn(III) respectively. The stability constant of the complex was calculated[48] and were found to be $2.11 \times 10^5$ and $4.23 \times 10^4$lmol$^{-1}$ respectively in methods A and B.

Analytical data

Under the optimized experimental conditions, linear calibration graphs, obtained by plotting the absorbance values against the concentration of benzidine. The
systems obey Beer’s law in the concentration range 0.102-0.714 and 0-4 μg/ml of benzidine with molar absorptivities of 6.27×10⁴ and 3.52×10⁴ L/mol/cm and Sandell’s sensitivity values are 0.0029 and 0.005 μg/cm² respectively for method A and B. The optical characteristics and the results of statistical analyses of the experimental data such as linear regression equation along with correlation coefficient, slope and intercept are summarized in TABLE 2. The correlation coefficients are found to be 0.9990 and 0.9978 for methods A and B, respectively, indicating the good linearity of both the calibration graphs with almost zero intercepts.

**Precision and Accuracy**

The precision and accuracy of the proposed methods (methods A and B) were evaluated by performing five replicate determinations of benzidine in pure form at three different concentrations following the recommended procedures. The precisions of the methods as expressed by the calculated relative standard deviations were found to be 0.95% and 1.04% for benzidine (0.31 and 2μg/ml in methods A and B respectively), whereas the corresponding accuracies as expressed by the calculated relative error were found to be ±0.51 and ±1.5% respectively for methods A and B.

**Reaction sequence**

As found in literature benzidine is oxidized to benzidine diimine, a colored radical cation, either with chloramine-T and N-bromosuccinimide[38] or with manganese dioxide[39] or with cobalt(III)- perchlorate[40]. Diimine is known to show different colors in acidic and basic media[49]. At pH lower than 3, yellow color dication BZ²⁺ is the main species showing maximum absorption in the region 400-440nm. And at pH =9 blue color radical cation BZ⁺ is the main species with λmax 600nm[49]. It is therefore believed that in these methods also cerium(IV)(method A) and electrolytically generated Mn(III) (method B)[25] oxidize benzidine to benzidine diimine chromophore in acidic medium. Then, Ce(III)[47] (method A) and Mn(II)[51] (method B) are expected to form complex anions,[Ce(SO₄)₂⁻] and [Mn(SO₄)₂⁻]²⁻ respectively and form ion-pairs with the chromophoric cation. The ion-pairs so formed, are appeared to be providing exceptional color stability to the systems with λmax 430nm and 410nm respectively in methods A and B. Therefore, from the literature background[49,38-40] and also on experimental results obtained here, the following reaction mechanism is given

**Effect of foreign ions**

For understanding the reaction selectivity, interference of common ions, which often accompany benzidine, for example, K⁺, Na⁺, NO₃⁻, NO₂⁻, Mg²⁺, SO₄²⁻.
Cl-, p-nitroaniline and p-anisidine solutions were added and investigated for the determination of benzidine under optimum conditions as described in the recommended procedures. The criteria for interference were fixed at an error of less than ±5%. The tolerance limit of the foreign ion tested for both the methods are given in TABLE 3. Nitrite ion, p-nitroaniline and p-anisidine did not interfere up to 0.081, 0.05 and 0.09 ppm respectively in method A and 0.01, 0.01 and 0.05 ppm respectively in method B. But when they were at higher levels cause a decrease in absorbance values as observed in both the methods.

TABLE 3: Effect of interference

<table>
<thead>
<tr>
<th>Foreign ion added</th>
<th>Tolerance limit in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method A</td>
</tr>
<tr>
<td>Na⁺</td>
<td>13.78</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.195</td>
</tr>
<tr>
<td>NO³⁻</td>
<td>0.081</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.770</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>8.928</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>20</td>
</tr>
<tr>
<td>K⁺</td>
<td>35</td>
</tr>
<tr>
<td>p-Nitroaniline</td>
<td>0.05</td>
</tr>
<tr>
<td>p-Anisidine</td>
<td>0.09</td>
</tr>
</tbody>
</table>

TABLE 4: Determination of benzidine in standard and industrial effluents

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount found(ppm± SD*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method A</td>
</tr>
<tr>
<td>Industrial effluents</td>
<td></td>
</tr>
<tr>
<td>Textile</td>
<td>0.013 ± 0.056</td>
</tr>
<tr>
<td>Paper</td>
<td>0.128 ± 0.047</td>
</tr>
<tr>
<td>Dye</td>
<td>0.051 ± 0.081</td>
</tr>
<tr>
<td>Standard taken (ppm)</td>
<td></td>
</tr>
<tr>
<td>0.202</td>
<td>0.197 ± 0.228</td>
</tr>
<tr>
<td>0.404</td>
<td>0.406 ± 0.311</td>
</tr>
<tr>
<td>0.607</td>
<td>0.586 ± 0.134</td>
</tr>
</tbody>
</table>

*Average of five determinations

Application of the methods

The new methods were applied for the determination of benzidine in industrial effluents and the results obtained are presented in TABLE 4. The results obtained from both the methods are highly reproducible, reliable and are in good agreement with the reported method[26].

CONCLUSION

The developed methods for benzidine are found to be rapid, simple, inexpensive and more sensitive with low values of relative standard deviations 0.95 and 1.04% respectively for methods A and B. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the developed methods without involving critical maintenance of experimental conditions. The reproducibility and reliability of the developed methods were also established by comparing the results of the new methods with that of a reported method[26]. The new methods do not require sophisticated instruments[10-24], extraction and pretreatment[26-31] of benzidine prior to its analysis. The developed methods are also sensitive enough to enable quantitation of benzidine at low concentrations. Hence, the developed methods were effectively employed for the determination of benzidine present either in pure form or in industrial effluents.

REFERENCES


\[
\begin{align*}
\text{NH}_2 + 2X & \xrightarrow{\text{H}_2\text{SO}_4} \text{NH}_2 + 2Y \\
\text{NH}_2 & \xrightarrow{Y + 2(\text{SO}_4)²⁻} \text{Z}⁻ \quad \text{n=2 for method A} \\
\text{NH}_2 & \xrightarrow{X=\text{Mn}(\text{III}), Y=\text{Mn}(\text{II}), Z=\text{Mn}(\text{SO}_4)²⁻} \text{Z}⁻ \quad \text{n=1 for method B}
\end{align*}
\]
Spectrophotometric determination of benzidine

Full Paper

Analytical Chemistry
An Indian Journal