Chapter 6.

Stable and highly sensitive Colorimetric Method for the Determination of Benzidine; involving ion-pair complexation.
INTRODUCTION:

Benzidine is one of the hazardous carcinogen\textsuperscript{1,2}, in addition it has the toxic effects causing dermatitis and cystitis in humans\textsuperscript{1}. It is a manufactured chemical compound that does not occur naturally. Benzidine is the trivial name for 4,4'-diaminobiphenyl in which two hydrogen atoms on biphenyl molecules are replaced by amines at para position. It is a white to slightly reddish crystalline powder turns dark on exposure to air and light, having melting point of 128° C\textsuperscript{3}. This compound decomposes on heating and reacts violently with strong oxidants, particularly with nitric acid. It is produced by the action of acids on diphenylhydrazine. Benzidine can be classified as arylamine in which one or more of the hydrogen atoms are replaced by aromatic groups such as homologues of aniline, p-aminobiphenyl, benzidine, and naphthylamine. Some arylamines are carcinogenic and the uses are restricted\textsuperscript{4}. Benzidine forms a blue precipitate with hemoglobin. Hemoglobin catalyzes the oxidation of benzidine to a polymer by hydrogen peroxide, which gives the blue color. This was the common screening test for occult blood\textsuperscript{5}.

Benzidine is the starting material in the dye manufacturing. Numerous substituted group on the benzidine ring system as well as amine groups offer important roles to each characteristic colour directly and in preparing next target colorants\textsuperscript{6}. It is important in azo dyes production due to strong fix to cotton. It and its derivatives have active applications in the synthesis of dyes, pigments, paints, rubber compounding agents and wide range of organic chemicals\textsuperscript{1-3}. Biphenyl structure of benzidine also provide the application in luminescence chemistry,
spectrophotometric analysis, molecular chemistry, and as a starting material for organometallic-complexes\textsuperscript{3,5,7}. The discharge of hazardous industrial and clinical wastes into natural water has become a serious environmental concern. With the growing concern over benzidine as an hazardous carcinogen and toxic compound\textsuperscript{1,2}, number of analytical methods for its determination are being reported\textsuperscript{1,2,8-11}. For example; titrimetry\textsuperscript{8}, spectrophotometry\textsuperscript{9}, chromatography\textsuperscript{10} and electrochemical method\textsuperscript{11}.

**LITERATURE SURVEY:**

A. Pielesz et al.,\textsuperscript{12} have described a method for the determination of some aromatic amines. The current environment-friendly regulations concerning textile products ban the marketing of textiles dyed with azo dyes capable of reductively splitting carcinogenic aromatic amines. The study analyzes seven azo dyes whose chemical structure determines various quantities of splitting aromatic amines, such as benzidine. For tests, seven commercially available azo dyes with aromatic amines in their structure were selected. These included two hazardous dyes: Acid Red 85 and Direct Blue 6, both capable of reductively splitting carcinogenic benzidine. Of the remaining five azo dyes, three—Ponceau SS, Sudan II, and Disperse Yellow 7—are capable of splitting \( p \)-phenylenediamine and aniline, while Mordant Orange 1 and Disperse Orange 3 can split only \( p \)-phenylenediamine. For Acid Red 85 and Direct Blue 6, the quantity of benzidine split from them was analyzed, depending on the conditions of the reduction process (e.g., in the HPLC method, 104 g/kg of dye for reduction in NaOH, and 41 g/kg of dye for reduction
in acetate buffer). The spectrophotometric method proved useful for preliminary analysis of amine content in examined samples. Spectrophotometric analysis may be used to determine the total content of amines counted as aniline. A full qualitative and quantitative analysis of amines released from azo dyes is possible using high-performance liquid chromatography (HPLC).

Yan Zhu et al., have developed a method for the simultaneous determination of a range of aromatic amines using cation-exchange chromatography performed on a standard ion chromatography column using d.c. amperometric detection. The analytes separated were 2,4- and 2,6-toluenediamine (2,4- and 2,6-TDA), aniline, o-toluidine, benzidine, p-chloroaniline, 4,4'-diaminodiphenyl (4,4'-DDP), m-nitroaniline and 1-naphthylamine. A Dionex CS12 column was used with gradient elution from an initial eluent of 5% CH₃CN+35 mM H₂SO₄ to 27% CH₃CN+35 mM H₂SO₄ (at 35 min). Detection limits in the range 2.6–22.6 µg/l were observed for all analytes except m-nitroaniline, for which the detection limit was 201 µg/l. Linear calibrations and good precision were observed and the method was applied to the determination of benzidine.

M. C. Nyman et al., have studied the 3,3'-dichlorobenzidine (DCB) and its degradation products, 3-chlorobenzidine (MCB) and benzidine, are of environmental concern because of their carcinogenic nature. The suitability of a small Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer for the analysis of these environmental contaminants in different types of matrices was explored. All the measurements were carried out by depositing the sample solution
directly on a disk that was introduced into the mass spectrometer. This approach is very fast and simple because it requires no prior chromatographic separation or derivatization. Calibration curves determined by collecting 70-eV electron ionization mass spectra of neat samples yielded lower limits of detection of 29 and 17 pg (total amount on the solids probe) for DCB and benzidine, respectively (based on a signal to noise ratio of ≥2:1), while chemical ionization with ammonia resulted in lower limits of detection of 21 pg for DCB and 9 pg for benzidine (total amount on the solids probe). FT-ICR analysis of sediments collected from Lake Macatawa (Holland, MI) verified the presence of DCB in this complex, environmentally significant sample matrix. Laboratory experiments designed to probe biodegradation and photodegradation pathways showed that DCB undergoes sequential dehalogenation to yield MCB and then benzidine under exposure to microorganisms and under simulated tropospheric solar radiation. The ability of the FT-ICR to determine elemental compositions of compounds introduced as described above was demonstrated for one of the degradation products.

M. Bouzige et al.,\textsuperscript{15} have described a new extraction immunosorbent involving antigen–antibody interactions was coupled on-line to liquid chromatography for the selective extraction in aqueous samples of benzidine and congeners, widely used as intermediate compounds in the manufacturing of dyes and pigments. Due to the cross-reactivity of the antibodies for analytes with chemical structures closely related to that of the analyte used for immunization, the immunoextraction sorbent was shown to be able to extract aminoazobenzene and related azo dyes with good recoveries. The on-line coupling was optimized for the trace
Benzidine
determination of benzidine, dichlorobenzidine, aminoazobenzene and some azo
dyes with detection limits in the range 0.1 to 1 µg/l. The high selectivity of the
immunoextraction was shown by comparing the analysis of an industrial textile
effluent obtained using precolumns packed either with a non-selective polymeric
sorbent or with the anti-benzidine immunosorbent.

F-F. Hsu et al.,\textsuperscript{10} have reported an isotope dilution mass spectrometry method
using capillary gas chromatography / negative ion chemical ionization to quantitate
urine concentrations of benzidine (BZ) and of its acetylated metabolites \textit{N}-
acetylbenzidine (ABZ) and \textit{N},\textit{N}'-diacetylbenzidine (DABZ). Urine samples were
purified by solid-phase extraction columns, reduced with LiAlH\textsubscript{4}/THF, and
derivatized with pentafluoropropionic anhydride. The derivatives were measured
by selected ion monitoring relative to deuterium-labeled internal standards. A
detection limit as low as 0.5, 0.8, and 1.5 ppt for BZ, ABZ, and DABZ,
respectively, can easily be achieved. Urinary concentrations of ABZ substantially
exceed those of either BZ or of DABZ in workers exposed to BZ or BZ-based
dyes. This method has been successfully used to measure BZ, ABZ, and DABZ in
1.0-ml urine samples collected from workers involved in manufacturing BZ and
BZ-based dyes.

M. D. Peiperl et al.,\textsuperscript{16} have analysed the Samples from 67 manufactured lots of
FD&C Yellow No. 6 (Sunset Yellow FCF; Colour Index No. 15985) for combined
benzidine. These samples were selected from those submitted to the US Food and
Drug Administration for certification between October 1991 and December 1992
by 13 dye distributors. Dithionite was used to reduce any combined benzidine

124
present in the form of azo or disazo dyes to free benzidine. This reduction was followed by extraction, diazotization and coupling with 2-naphthol-3,6-disulfonic acid disodium salt (R salt). The total benzidine was quantified as benzidine-R salt disazo dye by HPLC with detection at 540 nm and a quantification limit of 10 ng benzidine/g FD&C Yellow No. 6. Of the 67 samples analysed, 34 were found to contain more than 10 ng combined benzidine/g. Of these, 30 samples were from one manufacturing company, including three of its subsidiaries. The level of combined benzidine found ranged from 11 to 104 ng/g, except for one sample containing 941 ng/g.

M. J. Prival et al.,\textsuperscript{17} have examined 53 samples of FD & C Yellow No. 5 (tartrazine; Colour Index No. 19140) for combined benzidine. These samples, which represent separate lots from 12 dye distributors, were submitted to the US FDA for certification between 28 February 1990 and 27 June 1991. A method was developed to reduce the dye matrix with dithionite so that combined benzidine present in the form of azo or disazo dyes would be converted to free benzidine. Reduction was followed by extraction, diazotization and coupling with pyrazolone T, and the total benzidine present was quantitated as benzidine-pyrazolone T disazo dye (BZPT) by HPLC with detection at 500 nm. The limit of quantitation for benzidine in FD & C Yellow No. 5 by this method is 5 ng/g. 25 samples of FD & C Yellow No. 5 were found to contain 7–83 ng/g of combined benzidine that was released by dithionite reduction, 23 of these samples were from the same manufacturer. The identity of the BZPT from two FD & C Yellow No. 5 samples was confirmed by spectral analysis using HPLC with a photodiode array detector.
Valerie M. Davis and John E. Bailey, have presented the data suggesting the presence of the aromatic amine benzidine as a combined impurity in the regulated color additive FD&C Yellow No. 5. The benzidine exists as an azo-dye constituent that forms from free benzidine impurities introduced during the manufacture of the color additive. The presence of combined benzidine is ascertained by sodium dithionite reduction of the azo bonds in the commercial color additive. The resulting reduction products are extracted with chloroform, and the liberated benzidine is determined by high-performance liquid chromatography (HPLC). The levels of benzidine determined by HPLC exceed those levels of benzidine accounted for by direct determination of free aromatic amines in the unreduced color.

Jiří Barek et al., have described the Conditions for the determination of benzidine, o-tolidine, o-dianisidine, 3,3'-diaminobenzidine and 3,3'-dichlorobenzidine by classical and differential pulse voltammetry at a glassy-carbon stationary or rotating electrode, in the concentration range $10^{-5}$–$10^{-3}M$. The mechanism of the oxidation is discussed and applications are presented for the determination of these chemical carcinogens and their mixtures either directly, or after preliminary separation by extraction or thin-layer chromatography.

John E. Bailey, Jr. and Catherine J. Bailey have determined the benzidine, aniline, 4-aminobiphenyl (4-ABP) and 4-aminoazobenzene at trace levels in the colour additive FD&C Yellow No. 5 by diazotization and coupling with disodium 3-hydroxy-2,7-naphthalenedisulphonate (R-salt). The products are separated and determined by reversed-phase HPLC. All four amines were found in a survey of
commercial colours. 4-ABP is determined with 4,5-dihydro-5-oxo-1-(4-sulphophenyl)-1H-pyrazole-3-carboxylic acid as the coupling agent. Calibration is done by spiking a reference commercial colour at several levels.

Naomi Richfield-Fratz et al.,\textsuperscript{20} have presented data are for the determination of parts-per-billion ($10^9$) levels of aniline, benzidine, 4-aminobiphenyl (4-ABP) and 4-aminoazobenzene in the regulated color additive FD&C Yellow No. 6. The determination involves chloroform extraction of the amines from the color additive, followed by diazotization and coupling with the disodium salt of 3-hydroxy-2,7-naphthalenedisulfonic acid (R-salt). The coupling products are then analyzed by reversed-phase high-performance liquid chromatography.

Pratima Verma and V. K. Gupta,\textsuperscript{21} have described a method based on diazotization, coupling with α-naphthol in acidic medium, and extraction of the violet dye formed in alkaline solution into 3-methyl-1-butanol. Beer's law is obeyed up to 0.25 µg ml$^{-1}$ at 535 nm. The molar absorptivity and the Sandell sensitivity are $6.6 \pm 0.02 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ and 0.0028 µg cm$^{-2}$ respectively.

F. K. Kawahara et al.,\textsuperscript{1} have considered factors in the development of a method for the determination of toxic, water-soluble benzidines, involved providing a good technique for their adequate recovery, converting quantitatively the isolated amines to relatively stable derivatives suitable for measurement and storage, coupled with relative ease of preparation of the derivatives, and resolving the prepared compounds during their measurement. Benzidine and dichlorobenzidine are quantitatively extracted from wastewater and measured quantitatively through the preparation of their respective pentafluoropropionamides.
Benzidine (PFP) by using PFP-imidazole. Overall recovery efficiency for benzidines from wastewater ranges from 91 to 103%. These derivatives are relatively stable compounds and have detection limits of 0.2 pg or less, when electron capture detection is used with gas chromatography.

J. Barek et al., have studied the oxidation of benzidine, o-tolidine, and o-dianisidine with chloramine T and N-bromosuccinimide, the stoichiometry was established, and a mechanism was also proposed. An extraction-photometric determination of these diamines was developed, based on the reaction with excess chloramine T or N-bromosuccinimide and the measurement of the absorbance of the \( \text{N,N}'\)-dihalogeno-diphenoquinonediimines formed, after their extraction into chloroform.

A. Berka et al., have studied the oxidation of benzidine, \( o,o'\)-tolidine, and \( o,o'\)-dianisidine by manganese dioxide in an acidic medium. It has been shown that the studied substances are quantitatively oxidized by the reagent to the corresponding quinonediimines and that this reaction can be utilized for the indirect titrimetric determination of benzidine and \( o,o'\)-tolidine, based either on ferrometric titration of unconsumed manganese dioxide or on ascorbinometric titration of the quinonediimine formed.

Vladimír Dvořák et al., have studied the polarographic behavior of benzidine, \( N,N,N',N'\)-tetramethylbenzidine, 1,4-phenylenediamine, \( N,N,N',N'\)-tetramethyl-1,4-phenylenediamine, \( N,N'\)-diphenyl-1,4-phenylenediamine, \( N,N'\) — bis — 4 — dimethylaminophenyl — 1,4 — phenylenediamine, 4 — dimethylamino-N-methyldiphenylamine, 4-dimethylaminodiphenylamine and 4-aminodiphenylamine
Benzidine

in acetonitrile medium on the rotating platinum electrode and the possibility has been shown of utilizing the oxidation wave of these substances for their polarographic determination, taking into account the influence of water in the solvent and the acidity of the medium. Coulometric generation at constant current has been used to investigate the number of electrons exchanged in the reaction. The products formed in the electrode reaction are discussed.

R. M. Riggin and C. C. Howard\textsuperscript{23} have described a HPLC method for the determination of benzidine, 3,3'-dichlorobenzidine and 1,2 diphenylhydrazine in aqueous media. These compounds can be assayed either by direct injection or by solvent extraction or resin adsorption of the aqueous sample prior to the analysis with detection limits of less than 1\,\mu g/L. Linearity, precision and specificity of the method was excellent.

It appears from the literature that the chromatography\textsuperscript{1,13,15,17,18,20,23} is the most extensively employed method for the determination of benzidine, but the instrument is not widely available and costly too. Even some of the reported spectrophotometric methods\textsuperscript{9,21} also suffer from limitations like stability,\textsuperscript{9} necessitates extraction into organic solvent and strict maintenance of pH.\textsuperscript{9,21}

Manganese metal was isolated by Gahn in 1974. This can be prepared commercially either by the electric furnace process, the thermite method in which manganese oxide is treated with the metallic aluminium or by the electro-deposition from a solution of the manganese sulphate solution.\textsuperscript{24} Manganese is a gray-white metal, resembling iron. It is a hard metal and is very brittle, fusible with difficulty, but easily oxidized. The oxidation states of manganese are from +1 to
Manganese is essential to iron and steel production by virtue of its sulfur-fixing, deoxidizing, and alloying properties.\textsuperscript{25} Steelmaking, including its iron making component, has accounted for most manganese demand, presently in the range of 85\% to 90\% of the total demand. Among a variety of other uses, manganese is a key component of low-cost stainless steel formulations and certain widely used aluminium alloys.\textsuperscript{24} It is also added to gasoline in order to reduce engine knocking. Manganese(IV) oxide (manganese dioxide) is used in the original type of dry cell battery. Manganese dioxide is also used as a catalyst. Manganese is used to decolorize glass (removing the greenish tinge that presence of iron produces) and, in higher concentration, make violet-colored glass. Manganese oxide is a brown pigment that can be used to make paint and is a component of natural umber. Potassium permanganate is a potent oxidizer\textsuperscript{26} and used in chemistry and in medicine as a disinfectant. Manganese phosphating is used for rust and corrosion prevention on steel.\textsuperscript{25} The +3 oxidation state is also known, in compound such as manganese(III) acetate, this is also a powerful oxidising agent\textsuperscript{25}. 

Ion-pair complexes with complex metal anions are extensively being used in the determination of organic\textsuperscript{27} and inorganic analysis\textsuperscript{28}. Mn(III) has been used as catalyst in the synthesis\textsuperscript{29} and in the chemical oxygen demand determination of some organic compounds.\textsuperscript{30, 31} It is also an established oxidizing agent, hence, it has been used in redox reactions for the determination of organic\textsuperscript{30, 31} and inorganic ions.\textsuperscript{32} This prompted to try for the effectiveness of the electrolytically generated Mn(III) as a new oxidizing agent in determining benzidine by colorimetrically involving ion-pair complexation.
SCOPE OF THE PRESENT WORK:

Benzidine is one of the hazardous carcinogen\(^1,2\), in addition it has the toxic effects causing dermatitis and cystitis in humans.\(^1\) Hence a great deal of interest has been generated concerning its potential health hazards. Therefore determination of benzidine is desirable from the standpoint of environmental chemistry, food chemistry, waste water treatment and drinking water quality assessment. Number of analytical methods for its determination are being reported.\(^1,8-11\) For example; titrimetry,\(^8\) spectrophotometry,\(^9\) chromatography,\(^10\) and electrochemical method.\(^11\)

Among such methods, spectrophotometric method using chloramine-T \(^9\) as an oxidant is the oldest, with good sensitivity and precision. But the color of the solution is not stable on exposure to the strong sunlight.\(^1\) Whereas HPLC methods\(^18,20,23\) necessitates the extraction to the organic solvent, the instrument is not widely available and also costly too. Whereas in titrimetric method using manganese dioxide\(^8\) as an oxidant is suitable only at higher concentration. To overcome the above mentioned limitations; a simple, sufficiently stable, highly sensitive, and most affordable colorimetric method to determine benzidine in the standard sample and industrial effluents is described here. However, the developed method is a redox reaction, and indeed there is interference of the reductants. Therefore, an interference effect of common reductants on the determination of benzidine was studied, and the tolerance limits of some foreign ions were also
Benzidine

established. The conditions required for the quantitative determination of benzidine are described and related analytical parameters are also calculated.

Experimental:

Chemicals:

All the chemicals used were of analytical grade and the distilled water was used for preparing the solution and for dilution.

Benzidine (Sigma-Aldrich chemie GmbH, Steinheim, Germany), sulfuric acid (E. Merck, Bombay, India), manganese sulphate, hydrochloric acid, sodium hydroxide, p-nitroaniline, pyrocatechol, p-anisidine, sodium chloride, potassium nitrate, sodium nitrite, sulfamic acid, calcium carbonate and magnesium sulphate (SD fine chemicals, Boisar, India),

Reagents:

Preparation of manganese(III) solution:

10 ml of 2M manganese sulphate solution was diluted to about 100 ml using 5M sulfuric acid and electrolyzed this solution for about 2 h. Concentration of the prepared Mn(III) solution was determined iodometrically and the solution was found to be 0.0163 M with respect to Mn(III). From this, an aliquot of 6.1 ml was further diluted in a 100 ml volumetric flask up to the mark with 5 M sulfuric acid to obtain 0.001M solution.
Preparation of standard benzidine solution:

Accurately weighed amount of 10 mg of benzidine sample was dissolved in a clean beaker containing about 15 ml of ethanol, the solution was transferred into a 100 ml volumetric flask. The beaker was washed with distilled water and washings were also transferred to the flask. Then the solution was diluted to the mark with water. The prepared solution is of 100 ppm, from this solution an aliquot of 10 ml was further diluted with water to 100 ml to obtain 10 ppm standard solution.

Apparatus:

A Hitachi 2000 (Tokyo, Japan) UV-VIS Spectrophotometer and Elico SL 171 mini spec (Hyderabad, India) with 1 cm matched quartz cells were used for absorbance measurements.

Procedure:

Construction of calibration graph:

A series of labeled 10 ml volumetric flasks were arranged. A known but various volumes of 0.1-4 ml standard benzidine(10 ppm) solution was added to each flask. Then to each flask 0.5 ml of 0.001 M reagent Mn(III) solution added and the solution in each one of them was diluted to the mark with distilled water. The solutions were mixed well and kept at room temperature for about 15 min. The absorbance of the solutions were measured at 410 nm against the reagent blank, which was prepared like standard but without benzidine standard solution.
**Benzidine**

**Extractive preconcentration:**

A 100 ml portion of the solution (industrial effluents) containing benzidine was extracted with 100 ml of chloroform in two 50 ml portions, the combined extract contained in a 250 ml beaker was kept at room temperature in a fume wood till it becomes dry. The residue was dissolved in 10 ml of ethanol; the solution was transferred into a 25 ml volumetric flask. The beaker was washed with distilled water and washings were also transferred to the 25 ml volumetric flask and made upto mark using distilled water. Then 1.0 ml aliquot of solution was analysed as recommended in the procedure. The values obtained are given in the Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount found by the Developed method ppm* (±SD)</th>
<th>Amount found by the Reported method ppm* (±SD)</th>
<th>t-test</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industrial effluents;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Textile</td>
<td>0.013 (±0.003)</td>
<td>0.014 (± 0.005)</td>
<td>0.416</td>
<td>1.80</td>
</tr>
<tr>
<td>Paper</td>
<td>0.128 (±0.047)</td>
<td>0.132 (±0.083)</td>
<td>0.135</td>
<td>3.12</td>
</tr>
<tr>
<td>Dye</td>
<td>0.051 (±0.008)</td>
<td>0.053 (±0.007)</td>
<td>0.390</td>
<td>1.14</td>
</tr>
<tr>
<td>Standard; Taken (ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.105 (±0.037)</td>
<td>0.103 (±0.052)</td>
<td>0.085</td>
<td>1.98</td>
</tr>
<tr>
<td>0.5</td>
<td>0.493 (±0.047)</td>
<td>0.501 (±0.069)</td>
<td>0.270</td>
<td>2.16</td>
</tr>
<tr>
<td>2.0</td>
<td>2.010 (± 0.083)</td>
<td>1.970 (±0.108)</td>
<td>0.761</td>
<td>1.69</td>
</tr>
<tr>
<td>3.0</td>
<td>3.010 (±0.037)</td>
<td>3.060 (±0.053)</td>
<td>2.13</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Tabulated values at 95% confidence limit are (2.365) and (6.59) for t and F test respectively. *Average of five determinations.
Determination of stoichiometry and stability constant:

The composition between Mn(III) and benzidine was studied by modified Job's method of continuous variation.\(^3^6\) The concentrations of aqueous solutions of Mn(III) and benzidine were 0.0001M. Nine solutions were prepared containing Mn(III) and benzidine in various molar ratios so that their volumes were always amounted to 10 ml. The results obtained were used in plotting the graph, Fig. 1 that accounts for 2:1 stoichiometry between Mn(III) and benzidine respectively. Similarly, following the above procedure but with the solutions of 0.00005 M. The results obtained were used for the construction of the graph, Fig. 1 which again accounts for 2:1 stoichiometry between Mn(III) and benzidine respectively. The stability constant of the complex was calculated\(^3^6\) and it was found to be 4.23 X 10\(^4\) mol.\(^{-1}\)

![Graph](image)

Fig. 1. Determination of stoichiometry and stability constant.
Color stability of the experimental solution with time:

2.5 ml of the benzidine (10 ppm) solution was taken in a labeled 10 ml volumetric flask, 0.5 ml of Mn(III) solution was added and the solution was made up to the mark with water. Absorbance of the solution was measured at 410 nm against blank solution at different intervals of time as shown in Fig. 2, which indicate that, the color increases with time and attains maximum intensity at about 10 min and found to be almost stable for about 25 min. Afterwards, color intensity of the solution was found to decrease slowly with time, but retained its color for about 2 and 1/2 h. Though the solution prepared under specified experimental condition shown time dependent absorbance, however, this has not affected the results of the benzidine, since the standard and test solutions of benzidine were prepared almost at a time.

![Fig. 2. Time dependence of the reaction between benzidine and Mn(III).](image-url)
**Benzidine**

**Calibration graph in presence of sodium hydroxide and hydrochloric acid:**

To determine the effect of sodium hydroxide and hydrochloric acid on the results of the described method; three series, each series having four labeled 10 ml volumetric flasks were arranged. To the flasks of each series, measured but various volumes 1, 2, 3 and 4 ml of benzidine (10 ppm) solutions were added and only to each flask of the third series 1 ml of 0.04 M sodium hydroxide also added. To each flask of all the three series, 0.5 ml of the prepared Mn(III) solution was added. All the solutions of first series were diluted to the mark with 4N HCl and all the solutions of the second and third series were diluted to the mark with water. Absorbance of the solutions were measured after 20 min at 410nm. Calibration graph obtained by the experimental values was shown in the Fig. 3, which indicate that, the method could also be applied either in presence of dilute sodium hydroxide and dilute hydrochloric acid. The calibration graph constructed in presence of hydrochloric acid has shown the lesser molar absorptivity than in the calibration graph constructed in aqueous medium, because the rate of reduction increases with the increase in the hydrogen ion concentration\(^3\). Considering the sufficient absorbance and color sustainability of the solution which was diluted using water was preferred and also recommended in the procedure.
RESULTS AND DISCUSSION

The electrolytically generated manganese(III) reagent is considered to be oxidizing benzidine to yellow colored cation quinoned-imine. Thus the liberated Mn(II) was expected to combine with sulphate ions to form Mn(SO₄)₂⁻ anion, which would associate with quinonedimine cation to form ion - pair. The ion-pair formation appeared to be providing an exceptional color stability to the system. The yellow colored cation, quinoned-imine had shown the maximum absorbance in the 400 - 450 nm region and its absorption spectrum was shown in the Fig. 2, which agrees with the earlier observation. Absorption of the solution
Benzidine measured at 410 nm was found to be directly proportional to the amount of benzidine in the concentration range (0 - 4 ppm) and color of the experimental solution was found to be linearly stable for about 2 h at room temperature. Based on the experimental data optical characteristic values are given in Table 2 and also a probable reaction mechanism given.

Reaction mechanism:

\[
\begin{align*}
\text{Benzidine} & \quad \text{Quinonedi-imine} \\
\text{Mn(II) + 2 SO}_4^{2-} & \quad \text{Mn(SO}_4^2)^2 \quad 38
\end{align*}
\]
### Table 2. Optical Characteristics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave Length (nm)</td>
<td>410</td>
</tr>
<tr>
<td>Beer’s law Limit (ppm)</td>
<td>0 - 4</td>
</tr>
<tr>
<td>Molar absorptivity</td>
<td></td>
</tr>
<tr>
<td>(10^4 \text{ l mol}^{-1} \text{ cm}^{-1})</td>
<td>3.52</td>
</tr>
<tr>
<td>Sandel sensitivity</td>
<td></td>
</tr>
<tr>
<td>(\mu \text{g cm}^{-2} \text{ per 0.001A})</td>
<td>0.005</td>
</tr>
<tr>
<td>Regression equation ((y))</td>
<td></td>
</tr>
<tr>
<td>Slope ((b))</td>
<td>0.1935</td>
</tr>
<tr>
<td>Intercept ((a))</td>
<td>0.0000</td>
</tr>
<tr>
<td>Correlation co-efficient ((R^2))</td>
<td>0.9978</td>
</tr>
<tr>
<td>% Relative standard deviation ((n))</td>
<td>1.8</td>
</tr>
<tr>
<td>Stoichiometry</td>
<td></td>
</tr>
<tr>
<td>[Benzidine : Mn(III)]</td>
<td>1:2</td>
</tr>
<tr>
<td>Stability constant</td>
<td></td>
</tr>
<tr>
<td>(10^4)</td>
<td>4.32</td>
</tr>
</tbody>
</table>

\[Y = a + bx\] where \(x\) is the concentration of benzidine,

\(n = \text{six replicate analysis within linear range.}\)
Benzidine

OPTIMIZATION:
Effect of solutions used for the dilution, on the color stability and intensity of the experimental solution:

Three labeled 10 ml volumetric flasks were arranged. To each flask 2.5 ml of standard benzidine (10 ppm) solution was added. To each flask an aliquot of 0.5 ml of Mn(III) solution was also added. The first flask was made to the mark with distilled water, the second flask was diluted to the mark with 4N hydrochloric acid and the third flask was diluted to the mark with concentrated hydrochloric acid. Absorbance of each solution was measured at 410 nm against the respective blank. Absorbance of the solution I which was diluted with water was found to be 0.453 and the color sustained for about 2 h, whereas absorbance of the solution II which was diluted with 4N hydrochloric acid was found to be 0.435 and the color sustained for about 2 h and finally absorbance of the solution III which was diluted with concentrated hydrochloric acid was found to be 0.093 and the color sustained for about 30 min. Hence water was preferred over dilute hydrochloric acid and concentrated hydrochloric acid for dilution of the experimental solution to 10 ml.

Effect of foreign ions:

Tolerance limit of the common foreign ions namely, p-nitroaniline, pyrocatechol, p-anisidine, etc on experimental solution containing 2.0 ppm of benzidine was tested by studying the effects of these on the absorbance of the experimental solution. An error of less than ±5% in the absorbance values was considered to be tolerable. The tolerance limit of the foreign ions tested are given
in the Table 3. Nitrite and p-nitroaniline did not interfere up to 0.01 ppm level. At higher levels of it a decrease in absorbance was noted; however, interference up to 2 ppm of NO₂⁻ and 1 ppm of p-nitroaniline level was overcome by the addition of 1.0 ml 1% sulfamic acid to the sample before analysis. 

Table 3.

Effect of Foreign ions

<table>
<thead>
<tr>
<th>Foreign ion added</th>
<th>Tolerance Limit in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-nitro aniline</td>
<td>0.01</td>
</tr>
<tr>
<td>pyrocatechol</td>
<td>20.0</td>
</tr>
<tr>
<td>p-anisidine</td>
<td>0.05</td>
</tr>
<tr>
<td>K⁺</td>
<td>80.0</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>20.0</td>
</tr>
<tr>
<td>Na⁺</td>
<td>100</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>30</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>0.01</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>50.0</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>900</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>150</td>
</tr>
</tbody>
</table>
Absorption spectra of the 0.001M manganese(III) solution against water and the experimental solution containing 1.0 and 2.0 ppm of benzidine with 0.5 ml of 0.001M manganese(III) solution against blank in a final volume of 10 ml in the range of 350 -750nm was given in the Fig. 4.

Fig. 4 Absorption spectra of: a) Mn(III) reagent against water; b) 1.0 ppm benzidine with Mn(III) and c) 2.0 ppm benzidine with Mn(III) against blank.
The described method was applied for the analysis of benzidine within day and between the days. The results obtained vary by less than 2.0%. The described method was applied for the analysis of benzidine in the standard sample and industrial effluents. The optical characteristic parameters calculated from experimental data were given in Table 2. The reliability of the described method was tested by parallel determination of benzidine content in standard sample and industrial effluents using the reported method. The results obtained by both the methods are given in the Table 1. The reliability and reproducibility of the described method was concluded by F & t test values which are given in Table 1.

Conclusion:

Ion-pair complexes play very important role in organic and inorganic analyses. The ability of the metal anions to form ion-pairs with organic cations are many times used in the determination of metals and also the ability of orgonium ions to form ion-pair complexes with complex metal anions are extensively being used in the determination of organic and inorganic analysis.

Benzidine is one of the hazardous carcinogen in addition it has the toxic effects causing dermatitis and cystitis in humans. Hence a great deal of interest has been generated concerning its potential health hazards. Therefore elucidation of concentration of benzidine is desirable from the standpoint of environmental chemistry, food chemistry, waste water treatment and drinking water quality.
assessment. Number of analytical methods for its determination are being reported\textsuperscript{1,8-11}.

Among such methods, spectrophotometric method using chloramine-T\textsuperscript{9} as an oxidant is the oldest, with good sensitivity and precision. But the color of the solution is not stable on exposure to the strong sunlight\textsuperscript{1}. Whereas HPLC methods\textsuperscript{18,20,23} necessitate the extraction to the organic solvent, the instrument is not widely available and also costly too. Whereas in titrimetric method using manganese dioxide\textsuperscript{8} as an oxidant is suitable only at higher concentration. To overcome the above mentioned limitations; a simple, sufficiently stable, highly sensitive and most affordable colorimetric method involving ion-pair formation\textsuperscript{37} for the determination of benzidine in the standard sample and industrial effluents is described. Once prepared, Mn(III) reagent is stable for more than two months\textsuperscript{34} and it does not necessitate special care for the storage. The reliability of the proposed method was established by comparing the results obtained with the reported method\textsuperscript{21}. The standard deviation, t-test and F-test values calculated, Table 1 reveal that the accuracy and precision are affordable by the developed method. The developed method can serve either as an alternative or parallel to the reported method\textsuperscript{21} for the determination of benzidine in the pure sample and industrial effluents involving ion-pair complexation.
References:


2. King-Thom Chung, Ssu-Ching Chen and Larry D. Claxton, Mutation Research/Reviews in Mutation Research, 2006, 612(1), 58.


17. M. J. Prival, M. D. Peiperl and S. J. Bell, Food and Chemical Toxicology, 1993, 31(10), 751.


