Chapter IX

Clomipramine hydrochloride and trimipramine maleate as novel spectrophotometric reagents for the determination of N-bromosuccinimide and N-chlorosuccinimide in tap water and well water samples
CHAPTER NINE

Clomipramine hydrochloride and trimipramine maleate as novel spectrophotometric reagents for the determination of N-bromosuccinimide and N-chlorosuccinimide in tap water and well water samples 119 - 130

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Abstract

New, simple and novel methods for spectrophotometric determination of trace amounts of N-bromosuccinimide (NBS) and N-chlorosuccinimide (NCS) in tap and well water samples have been developed. The methods involve the oxidation of 3-methyl-2-benzothiazoline-hydrazono hydrochloride hydrate (MBTH) in hydrochloric acid medium by NBS or NCS and subsequent coupling with clomipramine hydrochloride (CPH) or trimipramine maleate (TPM) to yield blue colored derivative. The blue color derivative is having an absorbance maximum at 630 nm and is stable for 24 h. Beer's Law ranges, average molar absorptivity values, Sandell's sensitivity values and other analytical parameters are presented. Interference by various cations and anions has been investigated. The proposed methods have been applied to the analysis of NBS and NCS in tap and well water samples.

Keywords: N-Bromosuccinimide; N-chlorosuccinimide; spectrophotometry; 3-methyl-2-benzothiazoline-hydrazono hydrochloride hydrate; clomipramine hydrochloride; trimipramine maleate
IX.1. Introduction

N-bromosuccinimide (NBS) is perhaps the most important positive bromine containing organic compound. The compound was developed basically for use as a synthetic reagent but due to the high yield of the product in its reactions; it has been adapted for use in the determination of organic compounds. The applicability of the reagent is further enhanced by its high selectivity and by the diversity of experimental conditions under which it brings about any specific type of reaction, like substitution, addition, oxidation, etc. The reagent can be easily determined by iodometric titration as well as by certain indicator methods. While Ziegler, Djerassi, Wimkerlmann, Horner, Dauben and Wenkert, Mccoy, Waugh and several others studied the bromination reactions of N-bromosuccinimide, Reich and Reichstein, Fieser and Rajgopalan, Oliveto and Herzog and others pioneered the work on its oxidation reactions. The work on analytical applications of the reagent has been done during last two decades. NBS has been extensively used as a brominating and oxidizing reagent [1]. In aqueous solutions, its oxidizing properties were attributed to hypobromous acid generated by its hydrolysis. NBS has similar oxidation properties to hypobromite, but it is more stable.

N-chlorosuccinimide (NCS) is being used as an intermediate or a chlorinating agent in the synthesis of pharmaceuticals especially for tetracycline antibiotics (chlortetracycline, oxytetracycline, tetracycline, demeclocycline, methacycline, doxycycline and minocycline.). Carbon-carbon double bonds add chlorine and bromine rapidly in liquid phase. Halogen substitution reaction of alkenes should be carried out in the gas phase with halogenating reagents such as NBS and NCS. Allylic halogenations are carried out in the liquid phase.
Although NBS has been used in some chemiluminescence studies [2-10], most of them are applied to some organic species and pharmaceutical preparations; few are used in biological substance analysis. A wide variety of methods and techniques are available for the determination of NBS and NCS. These include titrimetric [11-13], potentiometric [14-16], voltametric [17], spectrophotometric [18] and flow injection chemiluminescence [19] methods.

In view of the above methods, novel and sensitive spectrophotometric methods based on the use of clomipramine hydrochloride (CPH) and trimipramine maleate (TPM) as chromogenic reagents in presence of 3-methyl-2-benzothiazoline-hydrazone hydrochloride hydrate (MBTH) as coupling reagent for the determination of N-bromosuccinimide and N-chlorosuccinimde, and their application in the determination of tap and well water samples have been reported.

IX.2. Experimental

IX.2.1. Preparation of reagent solutions

Standard solutions of NBS and NCS (1000 µg ml⁻¹) [S.D. Fine-Chem Ltd] were prepared by dissolving known amount in 1 liter of distilled water. Solutions of the required strength were prepared by diluting this stock solution with distilled water. Aqueous solution of MBTH (0.05% w/v) was prepared by dissolving 50 mg of the MBTH in 100 ml of distilled water; the solution was stored in amber bottle to protect from sunlight.
Chapter IX

Clomipramine hydrochloride samples

CPH and TPM were received as gift from Max Pharma, India. Solutions of CPH and TPM (0.1% w/v) were prepared in distilled water and a few drops of diluted sulphuric acid were added. Solutions of diverse ions were prepared by dissolving their respective salts. All other chemicals used in the analysis were of Analar grade.

IX.2.2. Apparatus and spectral characters

Specord 50 UV-vis spectrophotometer with 1.0 cm silica quartz matched cell was used for measuring the absorbance. The absorption spectra of the blue coloured complex with NBS and NCS showed maximum absorption at 630 nm. The reagent blank showed negligible absorption at this wavelength.

IX.2.3. Procedure for determination of N-bromosuccinimide

Appropriate volumes of standard solutions of

(i) NBS, 1.5 ml of 5N (v/v) sulphuric acid, 0.5 ml of 0.05% (w/v) MBTH and 1.5 ml of 0.1% (w/v) CPH or

(ii) NBS, 1.0 ml of 5N (v/v) hydrochloric acid, 0.5 ml of 0.05% (w/v) MBTH and 1.0 ml of 0.1% (w/v) TPM

were taken in a series of 25-ml calibrated standard flasks. After mixing the contents thoroughly the flasks were kept aside for 10 min. The solutions were made up to the mark using distilled water. The absorbance was measured against the corresponding reagent blank prepared under identical conditions but without NBS. Concentration of NBS in test solution was calculated from the regression equation computed using the Beer’s law data.
IX.2.4. Procedure for determination of N-chlorosuccinimide

Appropriate volumes of standard solutions of

(i) NCS, 2.0 ml of 5N (v/v) hydrochloric acid, 1.0 ml of 0.05% (w/v) MBTH and 2.0 ml of 0.1% (w/v) CPH or

(ii) NCS, 1.0 ml of 5N (v/v) hydrochloric acid, 3.0 ml of 0.05% (w/v) MBTH and 1.0 ml of 0.1% (w/v) TPM

were taken in a series of 25-ml calibrated standard flasks. The contents were mixed thoroughly and the flasks were kept aside for 10-15 min. The solutions were made up to the mark using distilled water. The absorbance was measured against the corresponding reagent blank prepared under identical conditions but without NCS. Concentration of NCS in test solution was calculated from the regression equation computed using Beer's law data.

IX.3. Results and discussion

The chemical reaction in the spectrophotometric study of N-bromosuccinimide and N-chlorosuccinimide, involves initially the reduction of NBS and NCS by MBTH and subsequent coupling of MBTH with CPH/TPM when a blue colour product is formed having $\lambda_{\text{max}}$ at 630 nm. The colour intensity remains constant for up to 24 h. The factors affecting the colour development, reproducibility, sensitivity and adherence to Beer's law were investigated by optimizing the analytical variables. A general reaction mechanism is proposed in Scheme IV.1.

During the course of study it was observed that the sequence of addition of reagents also influence to a great extent the intensity and stability of the colour. Less intense and unstable colour developed when the sequence of addition was
(i) CPH/TPM-acid-substrate-MBTH or (ii) substrate-acid- CPH/TPM-MBTH. But, when the sequence of addition was (iii) substrate-acid-MBTH-CPH/TPM and (iv) MBTH-acid-substrate-CPH/TPM more intense and stable blue colour was observed. This was expected as the reactions in sequence (i) and (ii) produced radical cation, while in (iii) and (iv) electrophilic substitution reaction was involved. Hence, sequence of addition (iii) was selected for further spectrophotometric investigations.

**IX.3.1. Optimization of analytical variables**

The concentrations and quantity of the reagent needed as also the reaction conditions were optimized and a standard procedure was adopted. Univariate procedure was followed for the optimization purpose.

**IX.3.2. Effect of reaction time**

The optimum time needed in the determination of NBS and NCS was determined. The blue colour formed in the reaction was not affected after 10 min. and remained constant up to 24 h. Therefore, 10 min was taken as reasonable time for the absorbance study.

**IX.3.3. Analytical parameters**

To determine the Beer's law limits, varying amounts of NBS and NCS were reacted with a fixed volume and concentration of MBTH and CPH/TPM under the definite acidic conditions and for specified time.
A linear correlation was found between absorbance at $\lambda_{\text{max}}$ for each substrate and concentration which in the range, is given in Table IX.1. Correlation coefficient, intercept and slope values, for the calibration data of the NBS and NCS are presented. The values presented show negligible intercept as by the regression equation, $y=ax+b$. The apparent molar absorptivities of the resultant coloured species are also presented in Table IX.1.

**Table IX.1:** Spectral data for the determination of NBS and NCS using MBTH as an electrophilic coupling reagent

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CPH (N-bromosuccinimide)</th>
<th>TPM (N-chlorosuccinimide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>630</td>
<td>630</td>
</tr>
<tr>
<td>Stability (h)</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Beer's law ($\mu$g ml$^{-1}$)</td>
<td>1.2 - 5.4</td>
<td>1.8 - 7.2</td>
</tr>
<tr>
<td>Recommended ion concentration ($\mu$g ml$^{-1}$)</td>
<td>3.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Molar absorptivity (L mol$^{-1}$cm$^{-1}$)</td>
<td>$1.24 \times 10^4$</td>
<td>$1.31 \times 10^4$</td>
</tr>
<tr>
<td>Sandell's sensitivity ($\mu$g cm$^{-2}$)</td>
<td>0.0214</td>
<td>0.0202</td>
</tr>
<tr>
<td>Regression equation $^*$:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (a)</td>
<td>0.0752</td>
<td>0.0545</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>0.2053</td>
<td>0.0428</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9915</td>
<td>0.9985</td>
</tr>
</tbody>
</table>

$^*$y = ax+b where x is the concentration of N-bromosuccinimide and N-chlorosuccinimide in $\mu$g ml$^{-1}$
IX. 3.4. Interferences

The selectivity of the proposed methods was studied by determining the effect of various cations and anions on the determination of NBS and NCS with MBTH-CPH/TPM method. An error of ± 3% was considered tolerable. Interference by foreign species was investigated by analyzing a standard solution of 2.0 µg ml⁻¹ NBS/NCS to which increasing amounts of interfering species were added. The tolerable limit of a foreign species was taken as a relative error not greater or lesser than 3%. The results showed that 400-fold excess of Mg²⁺, Zn²⁺, PO₄³⁻, SO₄²⁻, 200-fold of Al³⁺, Sr²⁺, NO₃⁻, 100-fold of Ba²⁺, Cd²⁺, Pb²⁺, Cl⁻, 50-fold of CH₃COO⁻, EDTA, F⁻ and tartrate did not interfere with the determination of NBS/NCS.

Table IX.2: Recovery of N-bromosuccinimide in water samples by proposed methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>NBS added (µg ml⁻¹)</th>
<th>NBS recovered by CPH method* (µg ml⁻¹)</th>
<th>NBS recovered by TPM method* (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>1.00</td>
<td>0.98 (98.0)*</td>
<td>0.99 (99.0)*</td>
</tr>
<tr>
<td>Well water 1</td>
<td>2.00</td>
<td>1.95 (97.5)*</td>
<td>1.96 (98.0)*</td>
</tr>
<tr>
<td>Well water 2</td>
<td>3.00</td>
<td>3.04 (101.3)*</td>
<td>3.03 (101.0)*</td>
</tr>
</tbody>
</table>

* - Average of three-determinations; * - Recovery in percentage

IX.3.5. Applications of the methods

The validity of the proposed analytical method was established by analyzing the natural water samples (tap and well water). The recommended procedures were applied for the determination of NBS and NCS after the addition to tap and well water samples. The results (Table IX.2 and Table IX.3) show that the methods can be
successfully applied for the analysis of both NBS and NCS in water samples containing these oxidants.

**Table IX.3:** Recovery of N-chlorosuccinimide in water samples by proposed methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>NCS added (µg ml⁻¹)</th>
<th>NCS recovered by CPH method* (µg ml⁻¹)</th>
<th>NCS recovered by TPM method* (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>1.00</td>
<td>0.98 (98.0)*</td>
<td>0.99 (99.0)*</td>
</tr>
<tr>
<td>Well water 1</td>
<td>2.00</td>
<td>1.97 (98.5)*</td>
<td>1.98 (99.0)*</td>
</tr>
<tr>
<td>Well water 2</td>
<td>3.00</td>
<td>2.97 (99.0)*</td>
<td>2.98 (99.3)*</td>
</tr>
</tbody>
</table>

* - Average of three-determinations; * - Recovery in percentage

**IX.3.6. Conclusion**

A first-ever use of N-substituted dibenzacepines involving electrophilic coupling reaction is proposed and the methods have been standardized for the spectrophotometric determination of N-chlorosuccinimide and N-bromosuccinimide. The proposed methods have unique features of simplicity, sensitivity and selectivity. The results invariably show that the reagents have clear positive features over most of the chromogenic reagents currently used for the purpose and the procedures show positive advantages over existing methods and the results are reproducible and the methods can be applied successfully for the determination of N-bromosuccinimide and N-chlorosuccinimide in tap and well water samples.
References


