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MINIATURIZATION OF A SPECTROPHOTOMETRIC ASSAY FOR AUTOMATED MONITORING OF INDUSTRIAL POLLUTANT "MORPHOLINE"

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ABSTRACT

During the last few decades, large-scale production and use of chemicals in various developmental activities have led to the release of huge quantities of hazardous substances such as morpholine into the environment. Morpholine is a precursor of carcinogenic nitrosamines. In general, most amines have high polarity, and so it is difficult to isolate them from water with conventional extraction methods such as the liquid-liquid extraction or adsorption-elution technique. Sensitive and selective analysis of determining morpholine at low concentration has not been reported. Hence, there is a need to develop an analytical technique, which should be efficient, economical and rapidly deployable for estimating minute ranges of this analyte. We herein describe a spectrophotometric method for quantification of morpholine based on its reaction with 1, 2-naphthoquinone sulfonate (NPQ/NQS). At room temperature itself morpholine undergoes a nucleophile substitution reaction in presence of sodium salt of 1,2-naphtoquinone-4-sulphonic acid under alkaline condition to form a red/orange product, namely 4-(4-morpholinyl)naphthalene-1,2-dione, which is detected at 480nm. The reaction is completed in 20 minutes in dark. A linear relationship existed between the absorbance and the concentration of morpholine with a regression correlation coefficient of 0.99. This is a small volume, simple,

sensitive, rapid, reliable and economical method for estimation of morpholine from 2 to 10 ppm and is amenable for automation to enable handling large number of samples at the same time.

Keywords: Morpholine, Nitrosamines, NPQ/NQS,4-(4-morpholinyl)naphthalene-1,2-dione Spectrophotometric method

INTRODUCTION

Environmental pollution (EP) is a global issue and the greatest problem that the world faces today. EP not only lowers the quality of life but also damages the environment and threatens human existence. Several factors and sectors are responsible for wide-spread environmental pollution. The ever growing problem is due to indiscriminate and deliberate release of hazardous and harmful substances. Although it is not easy for the scientific community to invent alternatives for total prevention of EP, but it can certainly provide solutions for constant and reliable monitoring, minimal discharge and/or effective remediation. The heterocyclic xenobiotic compound morpholine ($O(CH_2CH_2)_2NH$, Figure 1) is a colorless, oily, hygroscopic, volatile liquid with characteristic amine smell and of significant importance for different industrial applications [1]. Therefore, morpholine is present in effluents from several major industries e.g., petrochemical plants; rubber, paper, textile plating industry and dye & resin industry; coking plants, as well as many other pharmaceuticals industries viz., catalysts, antioxidants, bactericides, analgesics, anesthetics, and other physiologically active agents [2]. The large scale annual usage of morpholine (25000 tons/year)[3-4] and its wide range of applications are responsiblefor release of significant amounts of these chemical industrial effluents into water bodies. It is well known that morpholine reacts with nitrite to form carcinogenic nitrosamines [5-8]. Thus, removal of morpholine from dilute aqueous industrial effluents is a statutory obligation. Therefore, monitoring of morpholine in discharged waters is important for both protection of human health and the environment. The aim of this work was to develop an analytical method for quantitative estimation of morpholine which could be applied for sampling of wastewater and industrial effluents using an automated setup for large numbers of samples.

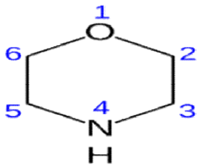

Figure 1: Chemical structure of morpholine (tetrahydro-1,4-oxazine) and 1, 2, 3, 4, 5 and 6 indicate the numberof carbon atom.

The methods available for estimation of morpholine involve chemical reactions that are characteristic of the individual functional groups. Many less sensitive and indirect methods based on titration [9] and chemical conversion to oxides (Chemical oxygen demand-COD) have been used in the past 2-3 decades. Several methods based on expensive analytical instruments such as Capillary isotachophoresis [10-11], Gas[12-13], High pressure liquid [14-16] and Ion/Cation exchange chromatography [17] are in use, but these cannot be deployed in the field. Each analytical method has its own advantage and limitation, e.g. cation-exchanger resins have a high capacity for amines, but they do not work well in the presence of electrolytes (e.g., NaCl, Na_2SO_4) [18]. These methods are time-consuming, tedious, and require sophisticated, expensive analytical instruments along with specific sample preparation steps. Spectrophotometric methods are most convenient because of their inherent simplicity, high sensitivity, low cost and wide availability indifferent settings. Spectrophotometric method described herein provides an opportunity to rapidly evaluate effluent composition/treatment methods for removal of morpholine adopted in industrial settings.

THEORY

This spectrophotometric method depends upon the nucleophilic substitution action of morpholine on the sodium salt of 1,2-naphtoquinone-4-sulphonic acid (NPQ/NQS) under alkaline condition [19-20]. A colored (orange/red) complex is formed after 20 minutes of incubation at room temperature, which is read at 480nm against a reagent blank (Figure 2). The rate of reaction, between morpholine ion andNPQ ion, is influenced by alkalinity of the reaction mixture and as it is a light sensitive reaction, appropriate precaution needs to be taken for accurate estimation of morpholine.

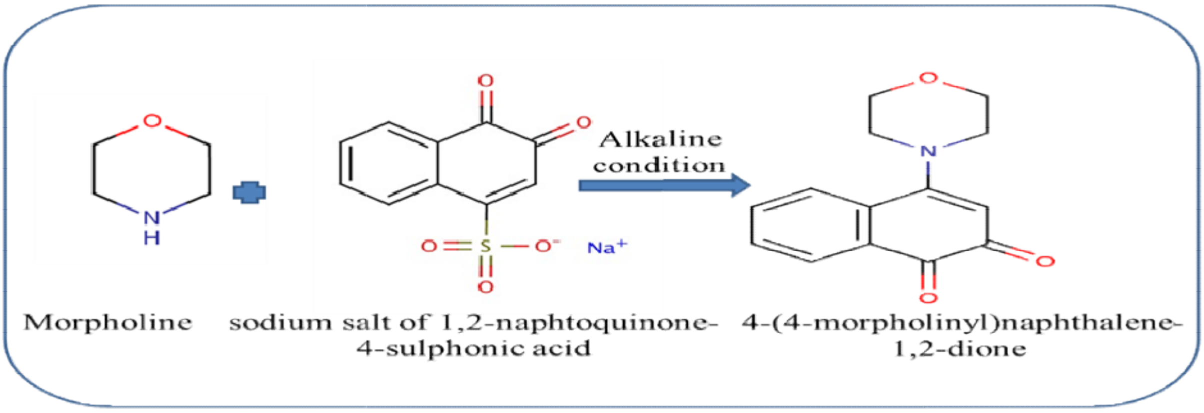

Figure 2: Nucleophilic substitution reaction of morpholine with sodium salt of NPQ/NQS

MATERIALS AND METHODS

Apparatus

An ELISA reader (ELX50/8MS BioTek India) provided with filters for specific wavelength was used to quantify concentration of morpholine. All calculations were done in Microsoft Excel for Windows.

Reagents and Solutions

Analytical grade morpholine and other chemicals were procured from Merck Ltd and Sigma-Aldrich, India. A standard solution of 1000 ppm (0.1% v/v) of morpholine was prepared in distilled water and further diluted 1:10 by dissolving 200 µl of 0.1% in 2.0 ml of double distilled water to obtain a working concentration of 100 ppm (0.01%). This was further diluted serially using distilled water to get a set of standard solutions. The stock solutions were stored in dark at room temperature. 0.115M (3%) of NQS reagent was prepared by dissolving 30mg in distilled water and making up the volume to 1ml. This solution was prepared fresh just before use and stored in dark at 4ºC. 1N sodium hydroxide (NaOH) was prepared by dissolving 4 gm in distilled water and made up to a final volume of 100 ml.

METHODOLOGY

Suitable aliquots (300µl) of morpholine working solution were pipetted into 8 wells micro-plate strips. 2 µl of 1N NaOH followed by 2 µl of 3% NQS reagent was added to each well. The reaction mix was left for 20 min at room temperature in dark. Absorbance was measured at 480 nm against a reagent blank and a calibration curve was constructed by plotting absorbance against morpholine standard concentrations (Figure 3). For effluent monitoring suitable aliquots of treated and untreated effluent samples were used. Using the calibration curve, the concentration of morpholine was calculated in the effluent sample/s.

RESULTS AND DISCUSSION

The present work describes a simple, precise and accurate spectrophotometric method for determination of morpholine by reaction of $-NH_2$ group of morpholine with a chromogenic reagent NQS under alkaline conditions. The method was found to be linear over a concentrationrange of 2-10ppm. The linear regression plot ($R^2 = 0.99$, slope of 0.0716) obtained with reference morpholine solutions is shown in Figure 4. While the earlier method reported by Stevens et al., 1990 [19] requires 50 ml sample volume, the present method requires only 0.3ml and is therefore best for handling a large number of samples using instruments for automation such asELISA plate reader and washer, etc. The new method was applied for morpholine estimation in treated and untreated effluents samples from a lyocell plant.

In lyocell process, N-Methyl Morpholine-N-oxide (NMMO) is used to the amount of ca. 140,000 tons/year [20-21]. NMMO based process comprises of dissolution and spinning of cellulose and nearly ~ 0.5% NMMO is discharged into effluent. NMMO undergoes reduction to N-methyl-morpholineand further demethylation to morpholine [21] (Figure 5).

The results in Table 1 show the sensitivity of the miniaturized assay and the specificity for morpholine with no reactivity with tertiary amine, NMMO. Sodium 1, 2-naphthoquinone-4-sulfonate reacts with both primary and secondary amines under alkaline pH at room temperature to give a chromogenic product with morpholine and not with NMMO.

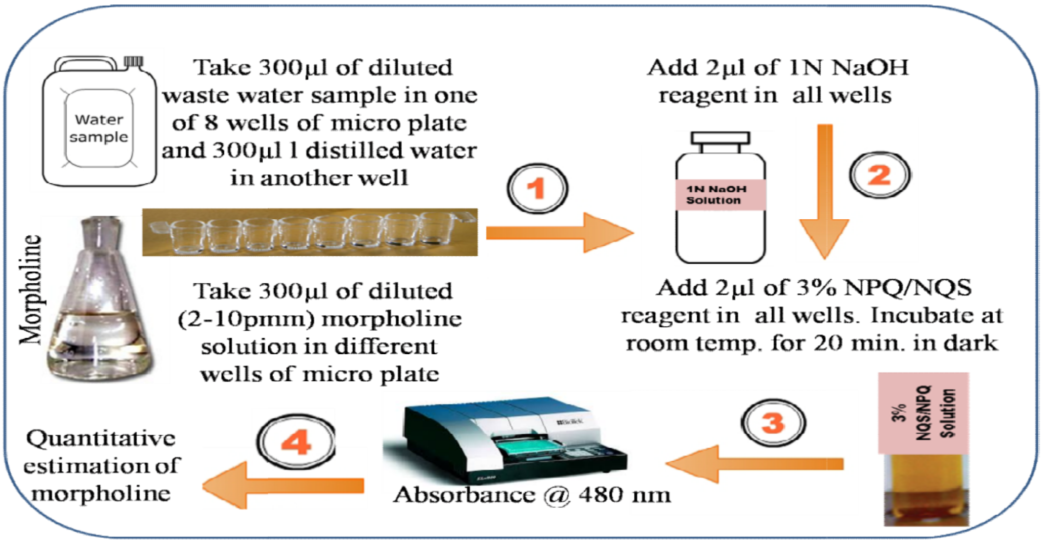

Figure 3: Flow sheet for the rapid spectrophotometric assay for morpholine estimation

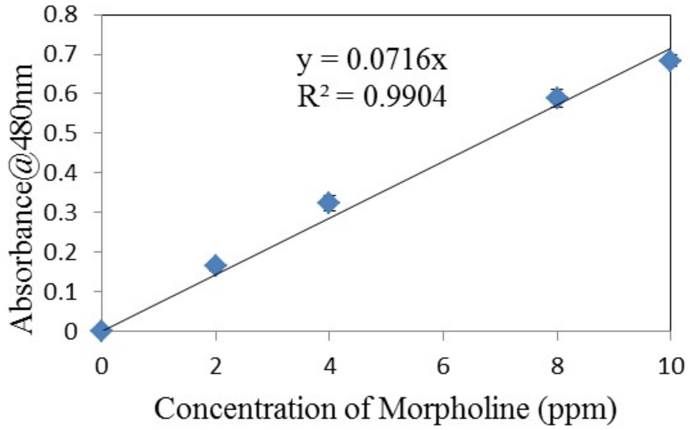

Figure 4: Standard curve for morpholine estimation

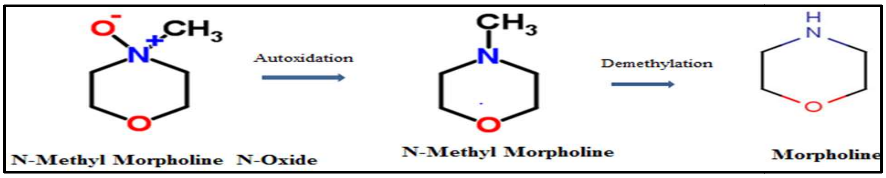

Figure 5: Fate of lyocell effluents in environment

Table-1 Detection of amines in untreated and treated effluent samples

Samples	Dilution required	Measured concentration	Actual concentration	Remark
Reagent blank	**Nil**	**---**	**---**	**--**
Untreated effluent	**1:1000**	**5.00 ppm**	**5000 ppm**	**--**
Treated effluent	**1:100**	**3.40 ppm**	**340 ppm**	**--**
Morpholine (0.1%)	**1:100**	**10.00 ppm**	**1000 ppm**	**2° amine**
Monoethanolamine (0.05%)	**1:100**	**4.50 ppm**	**500 ppm**	**1° amine**
NMMO (0.1%)	**1:100**	**Not detected**	**1000 ppm**	**3° amine**

CONCLUSION

The development of analytical methods for determination of pollutant amines have undergone and continue to undergo major changes. One of major target is to estimate these pollutants at trace levels preferably below 10ppm. Analysis of morpholine is of interest in biological and environmental chemistry due to its health hazardous nature. Even though several methods such as HPLC, chemical conversion to oxides (Chemical oxygen demand-COD) and GC have been used, but the described method is simple, sensitive and economical compared to all other reported methods and needs no sophisticated instruments. It is accurate, has good precision and desirable sensitivity for environmental monitoring. The present paper describes, for the first time, a method for estimation of morpholine at trace level with minimal sample volume and offers results comparable with those obtained with Gas chromatography (data not shown). In conclusion, this method can be a used for estimating the degradation of NMMO derivative, morpholine, in lyocell plant effluents in a reproducible manner.

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