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Characterization and application of azo dye (*E*)-*N*-phenyl-4-(thiazole-2-yldiazenyl)aniline (PDA) for biomedical sterilization

Ozge Surucu, Serdar Abaci*

Department of Chemistry, Faculty of Science, Hacettepe University, Beytepe, 06800 Ankara, Turkey

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ABSTRACT

Sterilization is the certain and absolute decontamination of microorganisms totally from all manner of alive and active species. Sterilization devices used in the sterilization processes are between laboratory and external patient application devices of biomedical device technology, and they are designed to remove equipments from germs. The application potential of hetarilazo indole based azo dyes in the biomedical sterilization are known and azo dyes come into prominence in this class because of simplicity of their synthesis and procurement of low-cost raw materials. In this work, the application potential of a novel synthesized azo dye, (*E*)-*N*-phenyl-4-(thiazole-2-yldiazenyl)aniline was investigated as an indicator in the biomedical sterilization performing the electrochemical and spectroscopic characterizations. The application of indicator was tested by ethylene oxide, hydrogen peroxide and peracetic acid sterilization techniques introducing into various sterilization rolls.

1. Introduction

The definition of sterilization has been passed into literature as "decontamination of environment from microorganisms in the extent to obtain an acceptable sterility assurance level" by AAMI (Association for Advancement of Medical Instrumentation) in 1995 (Zenciroğlu, 2008). At the same time, "Biotechnology for Health" field appears in the first sequences among science and technology branches where most resources are transferred in international standards, and this interesting field proceeds rapidly. Therefore, biomedical sterilization becomes more of an issue nowadays and various techniques are developing to meet such a demand.

A number of sterilization methods can be used for sterilization of equipment and materials properly. Ethylene oxide, hydrogen peroxide and peracetic acid sterilization methods get involved in chemical sterilization class, and they are widely preferred because of easy introduction into sterilization rolls (Milli Eğitim Bakanlığı, 2012).

Commonly used methods to control sterilization effectiveness are chemical control methods including chemical indicators. Such control methods must be applied according to "ANSI/AAMI/ISO 11140–1:2005 Sterilization of health care products – Chemical indicators – Part 1: General requirements" standard (Ataç, 2009). Azo compounds are among this class of indicators and can be used for sterilization. Absorption spectrums and voltammetric studies of azobenzene and its derivatives have been subjected to a certain number of investigations (Johnson and Florence, 1971; Menek et al., 1996; Menek, 1998). Although heterocyclic azo dyes are very important, a little attention has been showed for these compounds. Azo compounds play a vital role in electrochemistry depending on their colorization abilities, stabilities and selectivities into metal ions. Azo groups are electrochemically active and can be reduced on the surfaces of various electrodes, so a huge number of studies have been reported related with electrochemical reduction of azo compounds at various electrodes using voltammetric methods (Zanoni et al., 1999; Li et al., 2007; Chandra et al., 2008; Tian et al., 2013). However, electrochemical oxidation behavior has been studied less (Surucu et al., 2016, 2017; Surucu and Abaci, 2017). Therefore, the electrochemical behavior of a novel synthesized azo dye (*E*)-*N*-phenyl-4-(thiazole-2-yldiazenyl)aniline (molecular weight, MW = 280.3 g mol^{-1}) was investigated as an indicator for biomedical sterilization. The structure and color of PDA were shown in Fig. 1.

Light is the width of an electromagnetic spectrum includes all the wavelengths of light which are both visible and invisible such as radio waves, microwaves, infrared, ultraviolet, X-rays, and gamma rays. Intensity or brightness of a light source depends on the number of photons. The lower energy levels and longer wavelengths are brighter because they are excited more frequently, easier to drive an atom to less excited states. Therefore, more light colored photons are emitted than dark ones. In this manner, colorimetric techniques establish a good connection between wavelength and color, and are frequently used in the characterization of colorful materials.

In this work, the electrochemical characterization of a new azo dye, PDA was performed using simple cyclic voltammetry (CV) and

* Corresponding author.
E-mail address: sabaci@hacettepe.edu.tr (S. Abaci).

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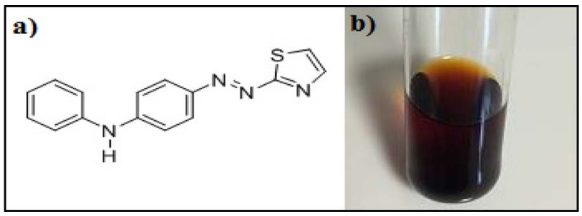

Fig. 1. a) The structure and **b)** color of PDA.

differential pulse voltammetry (DPV) techniques. The modification of disposable pencil graphite electrode (PGE) was made by PDA for the first time and the modified surfaces were characterized using attenuated total reflectance (ATR), ultraviolet-visible (UV–Vis) spectrophotometry and scanning electron microscope (SEM) techniques. At the same time, PDA was studied colorimetrically to determine the wavelength of PDA from the intensity of light. The azo dye was applied over sterilization rolls as an indicator after completion of characterization. Sterilization procedure was carried out following the color changes over rolls by ethylene oxide (EO), hydrogen peroxide (H_2O_2) and peracetic acid.

2. Materials and methods

2.1. Chemicals

As a supporting electrolyte, 0.1 mol L^{-1} of tetrabutylammonium perchlorate salt (TBAP) was purchased from Aldrich and prepared dissolving in 5.0 mL of dichloromethane solvent from Prolabo. The concentration amounts of PDA were used between 0.2 mg mL^{-1} and 1.0 mg mL^{-1}. 0.1 mol L^{-1} phosphate buffer solution (PBS) at pH 7.0 was prepared from pellet by dissolving in 100.0 mL of water. Sodium acetate and acetic acid were purchased from Merck to prepare 0.1 mol L^{-1} acetate buffer solution (ABS). Autoclave band (EN-ISO 11140-1 standardized package band, 19 mm × 50 m), Tyvek sterilization roll and big sterilization paper were procured from FTM Steriway firm and used for sterilization applications. Axis branded EO gas sterilization ampule set (EN ISO 13485, ARQ 5, ARQ 11, ARQ 20, ARQ 22 standardized sets) was purchased from SanMed Medical Device firm. Acetic acid and H_2O_2 stock solution (35% by volume) from Aldrich were used to prepare peracetic acid solution (40% by volume). Other reagents were in analytical grade. In all analysis, pure N_2 gas was passed from all solutions for sufficient period of time to remove oxygen.

2.2. Instruments

Electrochemical experiments were performed on *CH Instruments CHI660C* model potentiostat with a conventional three electrode system which consisted of pencil graphite working electrode (PGE), silver/silver chloride reference electrode (Ag/AgCl) and platinum counter electrode. PGE was prepared using mechanical pencil *Model T 0.50* (Rotring, Germany) as a holder for pencil lead (Tombo, Japan) which was purchased from a local bookstore and a metallic wire was wrapped around the metallic part of the pencil to provide electrical contact to the lead. All leads had a total length of 60.00 mm and a diameter of 0.50 mm. A total of 10.00 mm of lead was immersed in solution per measurement. Surface area of PGE in such a length was 15.90 mm^2. SEM images of the modified surfaces were obtained using a *Zeiss Evo 60 EP-SEM* scanning electron microscope. ATR analysis was carried out by *Nicolet IS 10* model device. UV–Vis absorption measurements were obtained by *UV–Vis Double Beam PC 8 Scanning Auto Cell UVD-3200, Labomed, Inc* model device.

2.3. Modification of PGE electrochemically

PGE was modified using CV technique with various number of cycles in 1.0 mg PDA dissolved 0.1 mol L^{-1} TBAP/dichloromethane solution between −1.5 V and +1.5 V vs. Ag/AgCl at a scan rate of 100 mV s^{-1}. The optimum thickness was chosen as 10 cycles and all PGE surfaces were prepared under these conditions. The influence of pH on the peak potential was investigated in a range of pH between 2.0 and 5.2 within 0.1 mol L^{-1} ABS. To discuss the electrocatalytic activity of PDA modified PGE, DPV technique was also used in various concentrations of PDA between 0.20 mg mL^{-1} and 1.00 mg mL^{-1} in 0.10 mol L^{-1} TBAP/dichloromethane solution between +0.80 V and +0.00 V vs. Ag/AgCl.

2.4. Characterization and application of PDA modified PGE

Surface dissimilarities of PDA modified and bare PGE surfaces were determined using ATR, UV–Vis and SEM techniques. ATR measurements were carried out in solid form of PDA. UV–Vis spectrum was obtained by 10 cycles PDA coated Indium Tin Oxide (ITO) electrode in 1.0 mg PDA dissolved 0.1 mol L^{-1} TBAP/dichloromethane solution between −1.5 V and +1.5 V vs. Ag/AgCl at a scan rate of 100 mV s^{-1}. SEM comparison was made using 10 cycles PDA coated PGE in 1.0 mg PDA dissolved 0.1 mol L^{-1} TBAP/dichloromethane solution between −1.5 V and +1.5 V vs. Ag/AgCl at a scan rate of 100 mV s^{-1} and bare PGE. The application of PDA as an indicator for biomedical sterilization was performed in various sterilization rolls including Tyvek, autoclave band and big paper. Several sterilization techniques such as EO, H_2O_2 and peracetic acid sterilizations were also applied to select proper surface and technique match.

3. Results and discussion

3.1. Determination of wavelength from the intensity of light colorimetrically

The color of a material is electromagnetic beams arriving our eyes in visible region between 380–760 nm. Complementary color is the combination of beam color hold by material and beam color forming visible color of material. Actual color of a material is the complementary beam color hold by material (Motor, 2003). The absorbed and appearing colors of materials were given with their light wavelength in Table 1. PDA appeared as red – purple colored, so it absorbed blue – green color as close to green. The wavelength of PDA was determined between 495 and 555 nm in visible region and the studies applied in this region.

3.2. Electrochemical behavior of PDA at PGE

1.0 mg PDA was dissolved in 5.0 mL of 0.1 mol L^{-1} TBAP/dichloromethane solution and the solution was scanned at PGE between −1.5 V and +1.5 V vs. Ag/AgCl at a scan rate of 100 mV s^{-1}. Cyclic

Table 1
Light wavelengths of absorbed and appearing colors.

Light Wavelength, λ (nm)	Region	Absorbed Color	Appearing Color (Complementary Color)
220–380	Ultra-Violet	Invisible	–
380–440	Visible	Violet	Yellow – Green
440–475	Visible	Blue	Yellow
475–495	Visible	Green – Blue	Orange
495–505	Visible	Blue – Green	Red
505–555	Visible	Green	Purple
555–575	Visible	Yellow – Green	Violet
575–600	Visible	Yellow	Blue
600–620	Visible	Orange	Green – Blue
620–700	Visible	Red	Blue – Green