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DEVELOPMENT AND CHARACTERIZATION OF POTENTIOMETRIC SENSOR FOR THE DETERMINATION OF ANTIOXIDANT PROPERTY OF WINE

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Abstract

A direct measurement of Gallic acid at the silver electrode modified with the help of anion exchange membrane (TDMAC) under different condition of cyclic voltammetery. The measured value of Gallic acid in the different samples of wine estimated the antioxidant property of wines, which depends on the oxidation process of Gallic acid. The presented modified method based on oxidation, which occurred on the working Ag electrode fabricated by anion exchange membrane. The modified sensor gives a linear response of potential with the Gallic acid concentration in the range from 0.05 to 3.0 g/L with best detection limit of 6.6 mg/L This sensor also gives a quick response time, satisfactory reproducibility, best sensitivity and long lasting stability, and measure the antioxidant capacity of the samples of wine without any interfering of ascorbic acid, metal ions and glucose. The results found by this modified electrode were correlated with the standard Folin- Ciocalteu method.

Keywords: Antioxidant property, wine, TDMAC, silver working electrode, Gallic acid.

Introduction

Gallic acid is a 3 ,4,5-trihydroxybenzoic white crystalline organic acid, which is present in tea leaves, different types of grapes, oak bark, wood, root, herbs and seeds [1]. GA is utilized in the food industry, dye industry (antioxidants and preservative), in the pharmaceutical industry as a food additive, cardiovascular disorder, anti-tumor, antiradial, anti-inflammatory and some for cancers disease [2-5]. It is used to decrease the possibility of disease, which grow up by slowing down or stop the oxidation process of the compounds, which occurred in the individual body, this is identified as oxidative stress [6]. Oxidative stress means to stop the growth of free radicals which causes the disease, has been associated with human diseases like cardiovascular disease, diabetes, Parkinson's, cancer, and Alzheimer's, [7,8]. Due to this property, the significance of GA in the wine has been increasing in the market. Gallic acid also give a valuable uniqueness to the wines due to their natural antioxidant property, so it is also a universal greatest sign to measure superiority and antioxidant property of wine [9].

Antioxidant property means the capacity of wine to destroy the free radicals, which cause diseases in the body. Many analytical methods are present for determining the total phenolic content and gallic acid concentration in the wines. High and Ultra performance liquid

chromatography (HPLC) [10], biosensor methods, mass spectrometry Folin–Ciocalteu (FC) method which depend on spectra [11], flow injection-chemiluminescence [12], nuclear magnetic resonance (NMR) spectral, and chemical analytical technique have been used to determine GA. But these all methods have some drawbacks like, expensive instrument, very sensitive, complicated procedure, necessity for monotonous sample preprocessing, and a high manufacturing price [13]. However biosensor is a best technique which used for determination of GA in the wine samples and their antioxidant property(red wine , white wine and rose wine), due to their high compassion, best detection limit, reproducibility, selectivity, low manufacturing cost, easy to handling, fast response and not complex procedure. [14–17]. They also give best authentic data about the analytes in sample compound's reaction [18-21].

Numerous electrochemical sensors have been developed which is based on carbon for measuring gallic acid in wine samples. One of them electrochemical biosensor Modified glassy carbon electrode (GCE) [22, 23] is a type of biosensor which depend on the fabricate of laccase attached with voltammetry developed effectively for fast measuring of GA and total phenolic content [24,25], potentiometric biosensor which depend on solid-contact was immobilized on the surface of GCE for the determination of total phenolic content and Gallic acid concentration in honey and propolis.

Modified-screen-printed electrodes modified by Au for measuring of Gallic acid in the green tea leaves samples [26], nanomaterials functionalized carbon-based composite electrodes by using cobalt oxide nanoparticles used for measuring the Gallic acid in wine samples (white wine and red wine) [27], and modified carbon paste electrodes (CPE) which based on the zinc oxide nanoparicles for a fast determination of Gallic acid [28, 29]. Carbon paste electrode modify by different metal oxide for the determination of particular analyte presented in the field [30, 31]. This is possible because of the less price come in the modification of carbon paste electrodes, best flexibility, reproducibility, simplicity of modification, quick response, less ohm resistance, easy to handling, less cost of the instrument, greatest surface area for the reaction, the widespread potential, and better electrochemical sensing properties, [32-33].

The presented potentiometric detection method depends on the movement of the ions through the ion selective electrode membrane tridodecyl methyl ammonium chloride (TDMAC). This is an anion exchange membrane which is more favorable for the movement of the specific ion. These specific ions react with the indicator ions, which released from the boundary of the membrane via the redox reaction, and lastly change the potential of the working electrode.

The potential change reflects the concentration of the substance measured in the sample [34, 35]. This method based on the permanganate ions, which release from the membrane boundary. This new presented method has some new application, for the measuring of GA concentration in the samples of wine (red wine , white wine and rose wine). Analysis conditions such as morphology of the membrane, optimized conditions like effect of temperature, pH, and electrochemical studies. The result comes by the presented biosensor correlate with standard data given by the Folin–Ciocalteu (FC) method.

Materials and Methods

Materials

Anion exchange membrane (TDMAC) was made from the chemicals, which procured online from Hi-Media Pvt. Ltd. Mumbai (India), Gallic acid (anhydrous) was obtained from (M.D.U) Pharmaceutical lab, model wine solution, wine samples procured from a local market in the town of Haryana, and all the chemicals which used in the experiment purchased from the pharmaceutical industry (Gurugram) were of best quality with no more refinement. The entire solutions use in the experiment was prepared with especially distilled water.

Instrumentation

Potentiometer (digital model LT-32, labtronic, India) with ORP Ag electrode was used for all electrochemical studies. Electronic weighing balance (Wensor), Ultra sonication was done with Chrom Tech

Ultrasonic Liquid Processor. For TEM JEM-2100F microscope was used, Varian 7000 FT-IR spectrometer was used for performing Fourier transform infrared (FTIR) spectroscopy. UV 2450 spectrophotometer was used for spectrophotometric measurement at Centre for Chemistry. The pH measurements were carried out by using a pH-meter with a combined pH reference electrode. All of the experiments were completed at room temperature 25^0 C.

Preparation of anion exchange membrane

The anion exchange membrane was made by the adding of 200 mg of 9 weight% TDMAC (tridodecyl methyl ammonium chloride), 31 weight% polyvinyl chloride, and 60 weight% o-NPOE and dissolve into the 2.0 ml of THF with a thickness of 210 mm. Before being put to use, the membrane mixture was first dry by using the sonification for ten minutes. It was then overflowed onto a tumbler disc with a thickness of 26 mm that was protected inside a glass plate. After that, it exposed in the air and dehydrated off entirely. After that, disc of membrane with a diameter of 6 mm were cut from the membrane [36].

Characterization of anion exchange membrane TDMAC

Anion exchange membrane TDMAC will be characterized by TEM (transmission electron Microscope). TEM is a microscopy technique in which electrons beam transmitted through the sample and forms the image. This image is formed after the interaction of electrons with the particle of the membrane and capable of giving higher resolution than light microscope. The thickness of the membrane is less than 100 nm and ultrathin is more favorable for this technique. UV spectrophotometer, which is based on the ultraviolet light or visible light interact with the particles of membrane and gives the modified spectra, and FTIR spectroscopy(Fourier transform infrared) is based on the radiation passed from the membrane, some radiations are absorbed by the sample but some passed out from this and recorded.

Cyclic voltammogram

Before the measurement of sample, the Ag working electrode dipped into the 0.1 M potassium permanganate solution. The modified silver electrode gives the change potential in between the range 0 to 1500 in mV with the potassium solution for the measuring of Gallic acid. Cyclic volatammogram noted in the range 0-1000 mV potential at changeable scan rate 5 to 200 mV s^{-1} at pH 2-7. After the optimization with these all parameter, the systematic curve found in between the concentration range of Gallic acid 0.05-3.0 g/L.

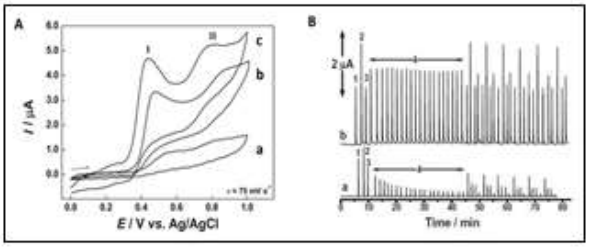

Figure: 1. Cyclic voltammogram shows that the oxidation of Gallic acid in a 1 M potassium permanganate solution at silver working electrode (a), bare Ag electrode (b), and modified Ag electrode with TDMAC (c) .

Sample Preparation for Gallic acid determination in wine samples

For the determination of Gallic acid in the different sample of wines like (red wine, white wine and rose wine), which procured from the local market and stored at the 4^0 C. We dilute the red wine 10 times before the use. A stock solution of 0.1M for Gallic acid was prepared with the model wine solution (4 g/L tartaric acid 12% ethanol at pH 2.5). 0.1M $KMno_4$ solution made every day for the experiment as a inner filing solution.

The Gallic acid found by this modified sensor compared with the folin-ciocalteu method. For making the FC standard graph we take 5.0 ml of white wine and 1.0 ml of red wine, and put into a beaker and make diluted to a final volume of 100 ml with doubly distilled water. After making this complete solution, we take 1.0 ml sample from this solution in another flask and add 0.5 ml of FC reagent, add doubly distilled water 2.5 ml, and lastly add 1.0 ml of sodium bicarbonate 20% vol. Later than, the absorbance was calculated at 734 nm, the Gallic acid concentration was determinate by

modified biosensor and FC method correlated by using a calibration curve [37].

Results and Discussion

Characterisation of TDMAC membrane

The anion exchange membrane prepared in the laboratory was characterized by the transmission electron microscopic (TEM) technique for checking their stability. The pictures of TEM show that the shape, size and dimension of membrane influence binding strength, releasing of ions make valuable, and dissolution of samples ions through the membrane become so easy. The pictures showing that the particles shape of anion exchange membrane were of semi-spherical and condensed, their range from 1nm to 100 nm represented. The particle size of the TDMAC membrane 100 nm shown in part (a) and 5 nm size of membrane particles shown in part (b) .

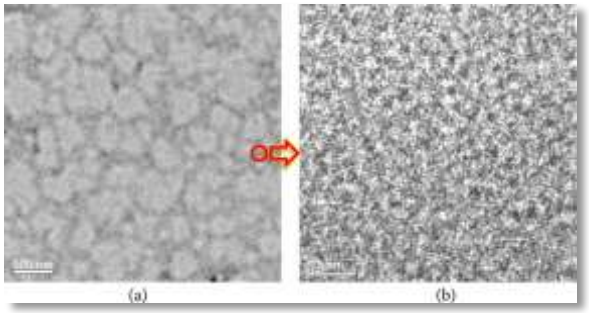

Fig:2. The TEM images of TDMAC membrane

Particle size and Zeta potential of TDMAC membrane

The size and zeta (stability) potential of TDMAC anion exchange membrane particles were analyzed by dynamic light scattering. The membrane particles size was found to have in between 10-100 d.nm. TDMAC membrane has a zeta potential of -28.3 mV (Fig 3), signifying membrane formulation stability.

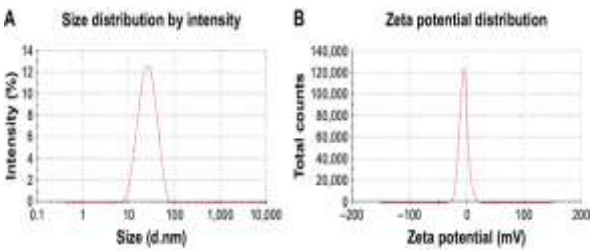

Fig: 3. Particle size image of TDMAC membrane (A) and Zeta potential of membrane (B)

FTIR spectra of TDMAC membrane

FT-IR spectra of TDMAC anion exchange membrane particles shows that the absorption peaks arrive in close proximity to in the range 1500 cm-1, 1000cm $^{-1}$ and at 500 cm $^{-1}$ in curve a, which are signature peaks of TDMAC. After fabrication of anion exchange membrane (TDMAC) on the silver working electrode provide a novel signature peak examined at 3500 cm-1, 3000 cm-1, 1500 cm-1, 1000, and at 500. An unreserved peak rose at 3500 cm-1 which is obtainable by the covalent bonding in between the particles of the membrane shown in the curve b given below.

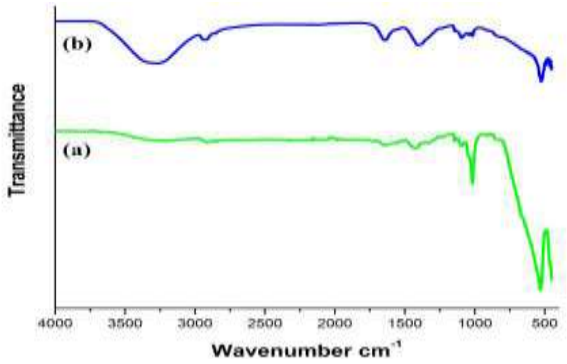

Fig: 4. FTIR spectra of anion exchange membrane (TDMAC)

Scanning Electron Microscopic (SEM) of fabricated electrode with TDMAC membrane

SEM image of the naked working Ag electrode examined that, a flat and unremarkable morphology. However, small granules and well lining in clustered form see on the surfaces of electrode (Fig.5) indicate of anion exchange membrane (TDMAC) on the exterior of silver working electrode smooth attached after fabrication. SEM images conform that the fabrication of anion exchanges membrane in a better way onto the working electrode.

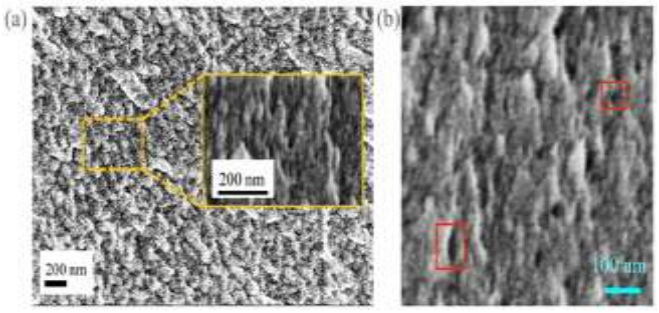

Fig: 5. The SEM images of TDMAC membrane (a) particle size of the membrane is 200nm (b) magnified image of membrane particle 100nm size

Electrochemical behaviour of Gallic acid at Ag working Electrode

The catalytic activities of anion exchange membrane (TDMAC) to regulate various electrochemical redox reaction processes. In electrochemical cells of redox reaction, anion exchange membrane deposit on solid surface of silver working electrode strength convey vary in current prototype as well as make possible novel sequence of potentiometric biosensors. The synthesized anion exchange membrane boundary was auxiliary characterized by Cyclic voltammograms (CV).

The method to estimate whether the anion exchanges membrane gain its catalytic effectiveness after being fabrication onto the working Ag electrode. The quantifications of membrane were functioned out in an electrochemically undisturbed redox cell. The voltammogram of the 0.05 g/L Gallic acid with potassium permanganate solution come at the modified electrode. Peaks instead of a chemical revolutionize of Gallic acid, when it react with potassium permanganate inner filling solution. After the redox reaction Gallic acid converted into semiquinone were analyze, with a peak value of +1.2 V at cathode and the parallel two peaks value at+ 0.95 V (peak 1) and +1.2 V (peak 2) at anode, this is happened due to electrochemical redox reaction created by membrane achievement. While organize same peaks, signified that the Gallic acid on Ag working electrode no ionic movement. Peak currents value arrived at anode and cathode by act of anion exchange membrane particles were distinguished at the scan rate 100 mV s^{-1}. Peak current ratio of cathode over anode was almost same. Difference shows in the peak value assessment with currents and the scan rate. TDMAC anion exchange membrane is an beneficial membrane which controlled the redox process in between Gallic acid and potassium permanganate on the electrode shown below.

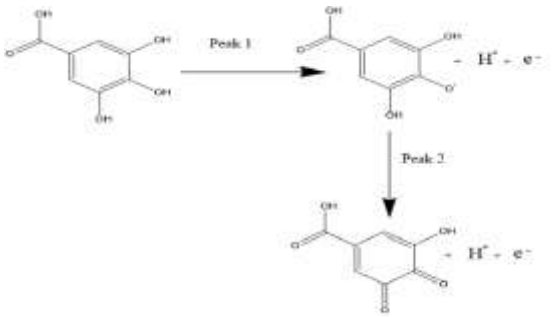

Fig: 6. Oxidation of Gallic acid on the electrode showing the two peak in CV

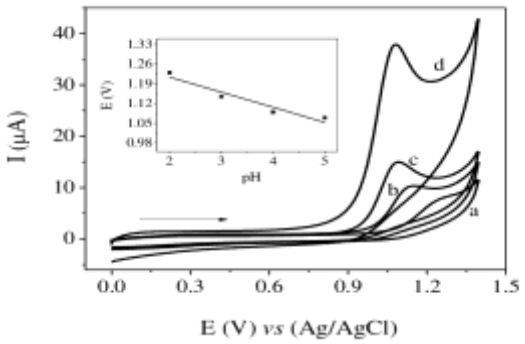

Figure: 7. Cyclic voltamogram of Gallic acid by using Ag working electrode at different pH values: (a) 2.0, (b) 3.0, (c) 4.0, (d) 5.0. Insert: effect of pH on the peak potential.

Optimization of Working Conditions of Biosensor

The stable pH of the TDMAC biosensor for the reaction was establish to be 2.5, which is more acidic as compared with free anion exchange membrane having pH 4.5, presentation comparison of pH 2.5 that the anion exchange membrane deposited on Ag working electrode. The assessment of stable pH alter is due to changed conformation of anion exchange membrane (TDMAC) after the fabrication on the working electrode. The sharp changing in the pH makes it additional supportive in determination of analyte. The stable and functional pH of the membrane biosensor was found to be 2.5, which is more favorable of gallic acid determination in wine, and no necessity of electrode pre-treatment.

The stable temperature for the best reaction founds to be 25 °C, which is a room temperature, for TDMAC action on the Ag working electrode found to be reasonably high than that of free membrane (20 °C). The response of the fabricated potentiometric sensor with anion exchange membrane in relation to changeable concentration of Gallic

acid was establish in linear range of 0.05–3.0 g/L and best detection limit is 6.6 mg/L. The reaction of the fabricated TDMAC in relation to changeable Gallic acid concentration was found in linearity with concentration range of 0.05–3.0 g/L shown below.

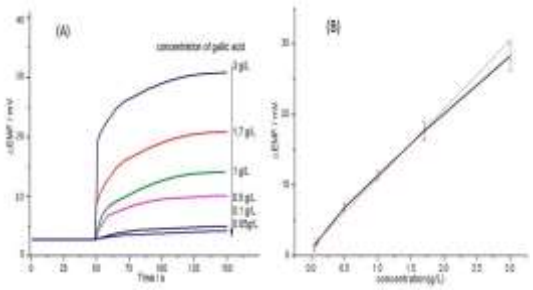

Figure: 8. Gallic acid concentration response of modified Ag electrode biosensor based on TDMAC membrane

Correlation

The selectivity of the present modified biosensor also was assessed by determination of total Gallic acid content in eight wine samples selected from the local market. A statistical evaluation of the results from this proposed biosensor and the Folin–Ciocalteu method is presented in Figure. The concentration of the total Gallic acid in the collected wine samples varied from 0.05g/L~3.0g/L. Additionally, the total phenolic content assessed by the proposed biosensor was higher as compared to the data obtained by the Folin–Ciocalteu method which shows that the proposed method determined the real polyphenol content. This is mainly because the present biosensor based on permanganate ion as an indicator also examined the other interfering substances like (sugars, glucose and ascorbic acid). Acceptably, the values of all test wine samples obtained from the two methods correlated highly and the Pearson correlation coefficient r was 0.6435. These results show that the proposed biosensor can be simple, capable and reliable tool for the rapid determination of total polyphenol content in wine samples.

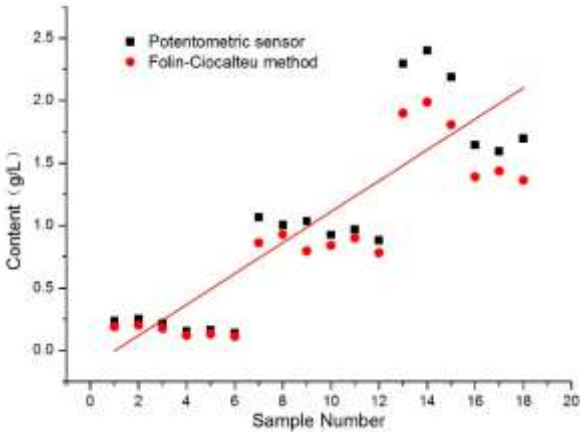

Fig: 9.Correlation of gallic acid in wine samples determined by potentiometric sensor and Folin–Ciocalteu method.

Storage capability of TDMAC membrane deposited on silver working electrode

Storage capacity of TDMAC membrane deposited on silver working electrode (Biosensor) was tested by every week over a period of 4 months. The Biosensor lost only 5 % of its initial catalytic activity, after its use over a period of 4 months, while storing at 4 °C. The better shelf life of Biosensor over previously reported sensors is due to the presence of TDMAC membrane deposited exhibit larger surface area and having strong affinity to covalently bind with the working electrode.

Conclusion

A novel biosensor for gallic acid was synthesized based on deposition potency of TDMAC anion exchange membrane on silver working electrode. They exhibit high substrate level of specificity, great sensitivity, and exceptional huge catalytic potency under ideal range of pH and temperature. Membrane is a key to run various industries for the production of potable water, chlorine-alkaline production and the treatment of industrial effluents etc. This Biosensor reveal improved performance in terms of response time (10 s), sensitivity (0.05–3.0 g/L gallic acid), best detection limit 6.6 mg/L, reusability (more than 100 times), and stability (4 months).

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