

Determination of 2,3,5-Triphenyltetrazolium Chloride Based on Flavinic Acid and 5-Nitrobarbituric acid Ion Pair Membrane

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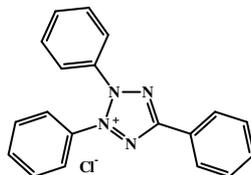
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A novel ion-selective membrane sensors consisting of flavinic acid/2,3,5-triphenyltetrazolium chloride (TTC) ion pair and 5-nitrobarbituric acid/TTC ion pair dispersed in a PVC matrix plasticized with dioctyl sebacat (DOS) is described. The electrodes shows a stable, near-Nernstian response for 1×10^{-2} – 5×10^{-6} M TTC at 25 °C with cationic slope of 56 ± 0.5 over the pH range 8-10. The lower detection limit and the response time are 5×10^{-6} M and 30-60 s, respectively. Selectivity coefficients for TTC relative to a number of interfering substances were investigated. There is negligible interference from many cations and anions. The determination of 0.5 – 200 mg mL^{-1} of TTC in aqueous solutions shows an average recovery of 99.0 % and a mean relative standard deviation of 1.4 % at 50.0 mg mL^{-1} . The direct determination of TTC in spiked wastewater and soil gave results that compare favorably with those obtained by the atomic absorption spectrophotometric method. The electrodes were successfully applied for the determination of TTC in wastewater and soil using potentiometric titration.

Key Words: PVC membranes electrodes, Ion selective electrode, 2,3,5-Triphenyltetrazoliumchloride, Flavinic acid, 5-Nitrobarbituric acid.

INTRODUCTION

Triphenyltetrazolium chloride (TTC) or simply tetrazolium chloride is a redox indicator commonly used in biochemical experiments especially to indicate cellular respiration. It is a white crystalline powder, soluble in water, ethanol and acetone but insoluble in ether. Triphenyltetrazolium chloride used for rapid detection of mesophilic anaerobic bacteria in the canning industry. Triphenyltetrazolium chloride and its compounds made their accurate determination is required. Molecular structure of triphenyltetrazolium chloride is shown below:



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In literature, various techniques *viz.*, carbon paste matrix^{1,2}, TCT-selective membrane sensor³⁻¹², fluorescence^{13,14}, electroreductions^{15,16}, colorimetric¹⁷⁻²⁴, chemiluminescence²⁵, flow injection (FI)-spectrophotometry^{19,26-40}, an enzymatic method^{41,42}, thin-layer chromatography⁴³⁻⁴⁸, radioactive microspheres^{14,49}, adsorptive voltammetry⁵⁰ are reported for the analysis of TTC. This work describes the preparation, characterization and some possible applications of a new triphenyltetrazolium chloride (TTC) sensors based on the use of flavinic acid hydrate (FA).

5-Nitrobarbituric acid or dilituric acid: 5-Nitro-2,4,6-trihydroxypyrimidine ion pair complex as the electroactive material and dioctyl sebacate (DOS) as a plasticizer. The sensitivity and stability offered by this simple electrode configuration are high enough to allow accurate determination of low levels of TTC. The selectivity coefficient measurements by many cations and anions are also negligibly small.

EXPERIMENTAL

All potentiometric measurements were made at 25 ± 1 °C with an orion (model 720) pH/mV meter. Double junction Ag/AgCl reference electrode was used. An orion electrode (model 90-02) filled with 10 % (w/v) potassium chloride was used in the outer compartment. Combination glass (ross pH) electrode (Orion model 81-02) was used for all pH measurements.

All chemicals were of analytical reagent grade unless otherwise stated and doubly distilled water was used throughout. Triphenyltetrazolium chloride (TTC) was obtained from Aldrich Chem. Co. (Milwaukee, WI, USA). Poly (vinyl chloride) powder (PVC) powder of molecular weight *ca.* 10000 was obtained from Aldrich Chem. Co. (Milwaukee, WI, USA). Tetrahydrofuran (THF) with a purity of 99 %, inhibited by 0.025 % butylatedhydroxytoluene was obtained from Aldrich Chem. Co. (Milwaukee, WI, USA). Dioctyl sebacate (DOS) plasticizer with a purity of *ca.* 99 % was obtained from Aldrich Chem. Co. (Milwaukee, WI, USA). *o*-Nitrophenyl octyl ether (*o*-NPOE) plasticizer with a purity of *ca.* 99 % was obtained from Aldrich Chem. Co. (Milwaukee, WI, USA). Dioctylphthalate (DOP) plasticizer with a purity of *ca.* 99 % was obtained from Aldrich Chem. Co. (Milwaukee, WI, USA). Triphenyltetrazolium chloride (TTC) (10^{-1} M) stock solution was prepared by dissolving 0.8370 g of TTC in 25 mL of (0.05 M) phosphate buffer solution (pH 9). Triphenyltetrazolium chloride (TTC) (10^{-1} – 10^{-6} M) standard solutions were prepared by appropriate dilution of the stock TTC solution with 0.05 M phosphate buffer solution of pH 9.

Flavinic acid with a purity of *ca.* 99 % was obtained from Aldrich Chem. Co. (Milwaukee, WI, USA). 5-Nitrobarbituric acid with a purity of *ca.* 99 % was obtained from Aldrich Chem. Co. (Milwaukee, WI, USA).

The following cations and compound solutions were prepared and standardized using the standard methods.

Dilute solutions (10^{-2} - 10^{-6} M) of these cations and compounds were prepared by 10-fold dilution of the stock solutions with 0.05 M phosphate buffer solution pH 9.

Sodium tetra phenyl borate as ion-exchanger and it is effect of response of ion-selective electrode membrane.

Preparation of TTC flavinic acid membrane: The electroactive material was prepared by adding 2 mg of flavinic acid to 10 mL (10^{-2} M) TTC. Complex was thoroughly mixed in a glass petri dish (5 cm diameter) with 200 mg dioctyl sebacate (DOS) as a plasticizer and 100 mg of poly (vinyl chloride) (PVC). The mixture was dissolved in a 5 mL of tetrahydrofuran (THF). The petri dish was covered with a filter paper and left to stand over night to allow slow evaporation of the solvent (THF) at room temperature. A master PVC membrane (about 0.1 mm thickness) was obtained.

Preparation of TTC, 5-nitrobarbituric acid membrane: The electroactive material was prepared by adding 2 mg of 5-nitrobarbituric acid to 10 mL (10^{-2} M) TTC complex was thoroughly mixed in a glass petri dish (3 cm diameter) with 200 mg dioctylsebacat (DOS) as a plasticizer and 100 mg of poly (vinyl chloride) (PVC). The mixture was dissolved in a 5 mL of tetrahydrofuran (THF). The petri dish was covered with a filter paper and left to stand over night to allow slow evaporation of the solvent (THF) at room temperature. A master PVC membrane (about 0.1 mm thickness) was obtained and glued to a polyethylene tube (3 cm length, 8 mm i.d.) using THF.

Preparation of TTC electrode: The PVC master membranes were sectioned with a cork pore (10 mm diameter and glued to a poly ethylene tube 2 cm length, 8 mm diameter) by using THF. A home made electrode body was used, which consisted of a poly ethylene, glass tube 8 cm length, 6 mm diameter, to which the polyethylene tubing was attached to one end and filled with the internal reference solution (1:1), (10^{-2} M aqueous TTC to 10^{-2} M KCl). An Ag/AgCl internal reference wire electrode (about 1.0 mm diameter) was immersed in the internal solution. The electrodes were conditioned by soaking in 10^{-2} M TTC solution for 9 h and stored in the same solution when not use.

Sensor calibration: The TTC-PVC membrane sensor was calibrated by immersion in conjunction with the reference electrode in a solution of 10 mL of phosphate buffer of pH 9 in a 50 mL beaker. After the addition of 1.0 mL aliquot of 1×10^{-2} – 1×10^{-6} M TTC solution with continuous stirring, the potential was recorded after stabilization to ± 0.2 mV and the EMF was plotted as a function of TTC concentration. The resulting calibration graph (Fig. 1) was used for subsequent determination of unknown thallium concentrations. Alternatively, the potentials displayed by TTC test solution before and after the addition of a 1.0 mL aliquot of known concentration of TTC were measured. The change in the potential readings was recorded and used to calculate the unknown TTC concentration in the test solution using the standard addition technique.

Effect of pH on the sensors response: The effect of pH on the potential readings of the two electrode systems was studied by immersing the ross combination glass electrode, PVC membrane sensor and a single junction Ag/AgCl reference electrode

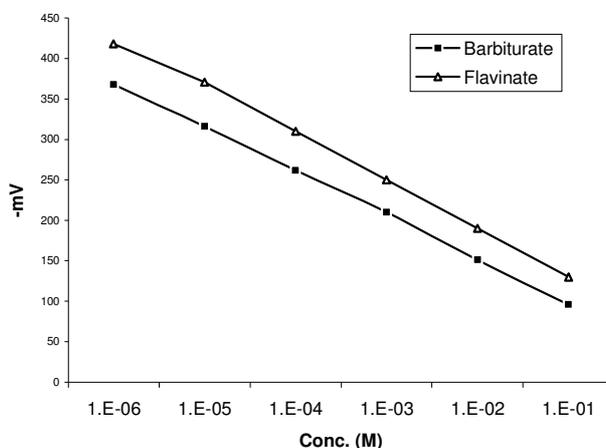


Fig. 1. Calibration curves of TTC sensors

in 50 mL beakers containing 25 mL aliquots of 10^{-3} - 10^{-4} M TTC aqueous solutions. The pH of each solution was gradually changed by adding small aliquots of concentrated sodium hydroxide and/or hydrochloric acid solutions. The potential reading at each pH value was recorded. The mV-pH profile of each TTC concentration was plotted for each electrode system.

Sensors selectivity: The potentiometric selectivity coefficients (K_F^{Pot}) of TTC sensors were measured by the separate solutions method^{51,52} in this method, the potentials of 1×10^{-3} M concentration of both TTC interfering species in 0.05 M phosphate buffer of pH 9 were determined. The selectivity coefficients were calculated using the Eisenman-Nicolsky equation⁵².

$$\log(K_F^{\text{Pot}}) = (E_1 - E_2)/S \quad (1)$$

where E_1 and E_2 are the potential readings observed after 1 min of exposing the sensor to the same concentration of TTC and interference, respectively and S is the slope of TTC.

RESULTS AND DISCUSSION

Nature and characteristics of TTC sensors: An ion pair is a compound or a complex which make some interaction with the experimental compound, an interaction may be hydrogen bond, vander Wall's forces, electrostatic forces, *etc.* The high insolubility of these complexes uses these compounds as electro active materials in membrane sensors due to rapid response 5-nitrobarbituric acid and flavinic acid with TTC were prepared, characterized and used as ion-exchange sites in PVC membrane sensors for TTC potentiometric response characteristics of these sensors under static were evaluated according to IUPAC recommendations. Response of detector for TTC test solutions of a wide concentration range. Calibrations based on peak height were almost linear in the range 10^{-1} - 10^{-5} M with slopes of 50 ± 1 mV/decade and 60 ± 1 mV/decade.

Potential response and stability of TTC membranes: Sensors based on 5-nitrobarbituric acid and flavinic acid with TTC ion pair complex membranes exhibited rapid response for various concentrations of TTC potential response time under stirred conditions was 5-10 s for increasing TTC concentrations from 1×10^{-5} to 1×10^{-1} M. The life-times of the sensors and detector were examined by repeated calibration every 2 days. Over a period of 30 days, no noticeable deterioration in the sensor performance in terms of detection limit, calibration curve slope and response time.

The sensors were calibrated by transferring 10 mL aliquots of 10^{-1} to 10^{-6} M aqueous solutions (10^{-2} M phosphate buffer solution at pH 9) of TTC to 50 mL beakers followed by insertion of the corresponding TTC-PVC membrane sensor and double junction Ag/AgCl reference electrode. The potential readings were recorded after stabilization to (0.2 mV and the EMF readings were plotted as a function of logarithm TTC concentrations. The calibration graphs were used for subsequent determination of unknown TTC concentration.

Table-1 summarizes the potential response characteristics of the proposed TTC sensors based on the means of 12 calibration runs carried out with three assemblies for each sensor during 6 months. These sensors exhibited Nernstian response with anionic slopes ranging from 55-60 mV per decade attributed to the presence of monovalent TTC cation. Calibration plots for sensors incorporating TTC-PVC ion pair membrane sensors responsive to TTC cation are shown in Fig. 1. The lower limit of the linear range, calculated as TTC concentration at which the slope of the calibration graphs deviated from linearity is 1×10^{-5} mol L⁻¹.

TABLE-1
RESPONSE CHARACTERISTICS OF TTC MEMBRANE SENSORS

Slope, (mV/decade)	57 ± 0.5	58 ± 0.5
Correlation coefficient, (r)	0.999	0.998
Lower limit or linear range, (M)	10^{-5}	10^{-5}
Lower limit of detection, (M)	10^{-5}	10^{-5}
Response time for 10^{-3} M(s)	30 ± 2	25 ± 2
Recovery time for 10^{-3} M(s)	50 ± 2	45 ± 2
Working range for 10^{-3} M(pH)	8.2-10.2	8.2-10.2
Life span, (week)	4	3

Effect of pH on the membrane performance for TTC and 5-nitrobarbituric acid ion pair complex membrane: The active anionic species amenable to potentiometric measurements is produced from TTC under basic conditions. Changes in the potential of TTC sensors as a function of pH were measured 10^{-3} - 10^{-4} M TTC solutions. From potential -pH profiles, it is evident that the optimum range of operation of the sensors is pH 8.2 to 10.2 for concentrations 10^{-3} - 10^{-4} M. Figs. 2 and 3 shows the effect of pH on the potential response of the sensors, other sensors display almost the same profile. The stability of sensors displayed in phosphate buffer solutions of pH 9.

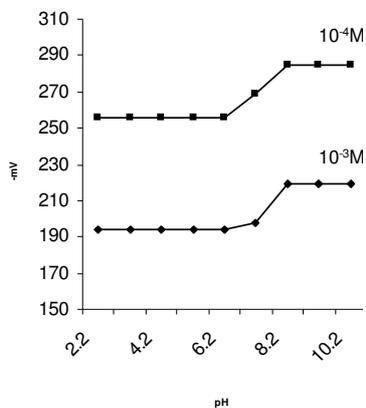


Fig. 2. Effect of pH on the potential response of TTC and 5-nitrobarbituric acid ion air complex membrane

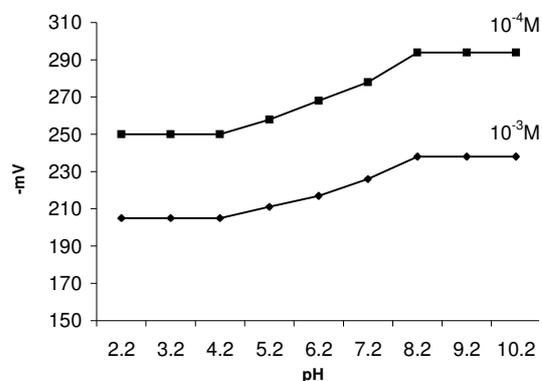


Fig. 3. Effect of pH on the potential response of TTC and flavinic acid ion pair pair complex membrane sensor

Effect of pH for TTC and flavinic acid ion pair complex membrane: Figs. 2 and 3 shows the pH-dependence of the observed potential of TTC membrane sensors for 1×10^{-3} - 1×10^{-4} M TTC test solutions. The potential response to the same concentration increase as the pH increased at pH range 6 to 9, with slopes 50 to 60 mV/decade attributed to the predominant presence of monovalent cation. The sensor response was fairly constant over the pH range 9 to 11, with slopes of 50 to 60 mV/decade. Measurement in basic media (pH = 9) offers the most appropriate conditions for sensitive and selective determination of TTC. Solutions within a rapid 10-fold increase in the TTC concentration (dynamic response) was 5 s.

Selectivity coefficients of TTC-PVC membrane sensors with respect to other organic and inorganic anions obtained by using the separation method at 10^{-3} M concentration are depicted in Table-2.

Application

In water: 1 mL aliquots of 10^{-1} - 10^{-6} M aqueous solutions (10^{-2} M phosphate buffer solution at pH 9) and TTC added to 50 mL beaker with 1.0 mL sample water. The potential readings were recorded after stabilization to (0.2 mV and the EMF readings were plotted as a function of logarithm TTC concentrations (Fig. 4).

In soil: A sample soil takes 5 g of soil in 50 mL 10^{-2} M phosphate buffer solution at pH 9. Transfer 1 mL aliquots of 10^{-1} - 10^{-6} M aqueous solutions of TTC to 50 mL beakers to 1.0 mL sample soil. The potential readings were recorded after stabilization to ± 0.2 mV and the EMF readings were plotted as a function of logarithm TTC concentrations (Fig. 5).

TABLE-2
 POTENTIOMETRIC SELECTIVITY COEFFICIENTS (K_F^{Pot}) OF (TTC),
 5-NITRBARBITURIC ACID AND FLAVINIC ACID ION PAIR
 COMPLEX MEMBRANES SENSORS

Compounds or cations	Barbiturate K_F^{Pot}	Flavinete K_F^{Pot}
Sodium chloride	3.0×10^{-1}	5.6×10^{-1}
Potassium chloride	6.9×10^{-1}	2.3×10^{-1}
Glucose (Dextrose)	8.7×10^{-5}	6.3×10^{-4}
Sucrose	3.9×10^{-5}	1.0×10^{-3}
Ammonium chloride	3.9×10^{-1}	5.6×10^{-1}
Magnesium sulfate	4.1×10^{-4}	1.7×10^{-2}
Urea	1.1×10^{-3}	4.6×10^{-2}
Arginin	2.5×10^{-3}	4.8×10^{-2}
Folic acid (vitamin B ₉)	2.7×10^{-2}	1.4×10^{-1}
Riboflavin (vitamin B ₂)	3.1×10^{-3}	4.6×10^{-2}
Aniline	5.2×10^{-5}	1.5×10^{-3}

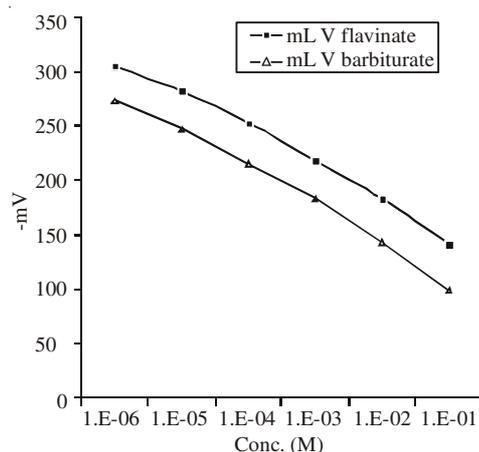


Fig. 4. Application of TTC in water

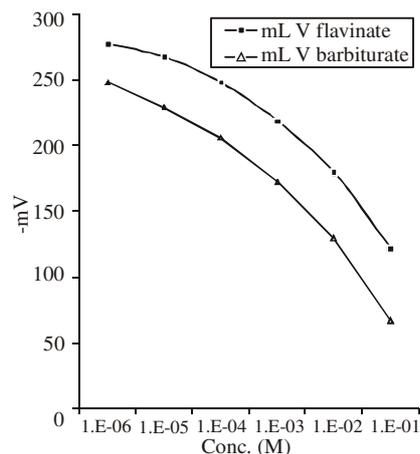


Fig. 5. Application of TTC in soil

Conclusion

Two new ion pair membranes sensors were suggested for the determination of TTC in pure, soil and water samples. A comparison between the performances characteristics of the sensors based on the use of PVC, dioctyl sebacate (DOS), DOS is plasticizer, sodium tetraphenylborate and sodium tetraphenylborate are ionic additive, 2 mg 5-nitrobarbituric acid with 10 mL (10^{-3} M) tetrazolium (tetrazolium barbiturate ion pair) and 2 mg flavinic acid with 10 mL (10^{-3} M) tetrazolium (tetrazolium flavinate ion pair) in form of classical ion pair membrane sensors were demonstrated. The results indicated that the two sensors are perfect sensitive sensors for determination of TTC, quantitative, range, life, time.

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