Amperometric Biosensor for Nitrate Determination Drinking Water: A Review

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ABSTRACT

Determination of nitrate in drinking water and biological fluids is important from biological point of view. It is formed by combination of nitrogen with oxygenated water. Standard concentration of nitrate in drinking water is 45 mg/l (EPA). Nitrate concentration in drinking water is varied to large extent due to human activities. Determination of nitrate with the nitrate reductase based biosensor is paid great attention for determination of nitrate in drinking water. A short categorization and description of materials commonly used for construction of electrode, e.g. platinum, glassy carbon, graphite, screen printed electrode, sol-gel, clay composite , polymeric membranes, and various strategies of enzyme immobilization e.g. physical binding, covalent binding, gel entrapment, electro polymerization and self assembled architectures are presented in this review. Determination of nitrate with suitable biosensor and further improvement in biosensor characteristics, are also presented.

Keywords: nitrate reductase (NaR), polymeric membrane, biosensor, nitrate determination, water and electrode.

1. INTRODUCTION

Determination of nitrate is important, particularly from biological and industrial point of view. It is formed by the combination of nitrogen from ammonia or other sources with oxygenated water. Nitrate is commonly found in water [11], waste water and soil [23], fertilizers, biological fluids [3], plant and animal tissue. In biological fluids concentration of nitrate is increased by oxidation of endogenous NO and exogenously by intake of food, vegetables and water. Most of the nitrate comes from vegetables (80%) and drinking water (20-25%) [14]. World Health Organization (WHO) and US Environmental Protection Agency (EPA) imposed a maximum permissible limit of 45 mg/l of nitrate in drinking water and in 1974, Congress passed safe drinking Water Act to set safe nitrate concentration in water i.e. 10mgN/liter (State & Federal laws). Level of the nitrate in drinking water is increased by various sources such as disposal of sewage, industrial waste related to food processing. Misuse of fertilizers on lawns close to shallow water and in crops, increasing the level of nitrate in ground water (source of drinking water). This increased level of nitrate in water more than 45 mg/l leads to serious health effects in human and animals this may cause

methaemoglobinemea in infants [5], increase blood pressure in school children, interference in thyroid gland function [9], affecting the utilization of vitamin A, watering of eyes, rough hair coat, reduced appetite, weight loss. Nitrate poisoning (bluish skin coloring around eyes and mouth) [12] and pancreatic and stomach cancer are serious health effect of nitrate caused due to formation of N-Nitroso compound in body [6]. For human health, removal of nitrate from drinking water is required, but it needs high cost due to solubility of nitrate in water.

2. REVIEW OF LITERATURE

Determination of nitrate in water samples and food stuffs is necessary due to health risk. It has been employing a range of analytical techniques including – Ion exchange chromatography [19], Ion interaction liquid chromatography [6], colorimetry, mass spectrometry[22], differential pulse Volta metric method [24], polarography and three wavelength method [15] etc. But these methods are either tedious or time consuming or not sufficiently specific.Now a day the concentration of nitrate could be determined using a biosensor which is simple & effective. Biosensor is device incorporating a biological molecular recognition component connected to a transducer that can output an electronic signal proportional to the conc. of analyze being sensed [16]. The area of biosensor particularly enzyme based amperometric and potentiometric electrodes had received great attention due to combination of advantage of electrochemical techniques with high substrate specificity of enzyme, quick response time and ease of procedure [25]. Nitrate Reductase (NaR) is an enzyme, mediates reduction of nitrate to nitrite and mainly used in nitrate biosensor. NaR is a metalloprotein and contains one each of Flavin (as FAD), heme iron, molybdopterin and belongs to a super family of enzymes including xanthine oxidase and nitrite reductase [13]. NaR is present in human in saliva, liver, plasma, in other animals and plant parts. It was purified from barley [18], spinach [17], corn, squash [5] and Aspergillus [8].NaR included in biosensor used for measuring of nitrate concentration were first reported in 1994. This reduction reaction by NaR is affected by oxygen, to reduce effect of oxygen; an oxygen scavenger is applied to a biosensor.

Reaction catalysed by NaR is

$$\text{Nitrate} + NADH^+ + H^+ + 2e^- \xrightarrow{\text{NaR}} \text{Nitrite} + NAD^+ + H_2O$$

Various biosensors have been used to determine nitrate level in drinking water. Main enzyme used in these biosensors was nitrate reductase.

First of all the electro polymerization of NaR-amphiphilic pyrrol-viologen mixture preabsorbed on electrode surface was investigated [5]. Immobilization of enzyme on viologen-polypyrrole film mediated by entraptment. The immobilized enzyme catalyzed the reduction of nitrate to nitrite

mediated viologen redox couple. The sensitivity and detection limit of this biosensor were 13.8 mA M^{-1} cm^{-2} and 4x 10^{-7} M, respectively. Further a nitrate biosensor based on ultrathin film composite membrane concept was developed for nitrate determination [16]. Composite membrane is prepared by electropolymerization of a thin anion permselective coating of (1– methyl 3– pyrrole–1– trimethyl) pyridinium across microporus support membrane. Nitrate was reduced enzymatically yielding oxidized form of nitrate reductase which is reduced by methyl-viologen, and then nitrate concentration is directly related to methyl-viologen reduction current. This sensor showed good sensitivity to nitrate, with a detection limit of 5.4µM and a dynamic range which extends upto 100µM nitrate. A nitrate biosensor on the basis of nitrate reductase (extracted from Pseudomonal stutzeri) was investigated [16]. Nitrate reductase immobilized on graphite electrode for nitrate determination in natural samples and use of resulting sensors to characterize the bioelectrochemical properies of mediators of phenothiazine, triphenyl methane, sulfanaphthaleine and viologen type dependence current densities on electrode potential.

Azure A gave the highest current density of 70µA cm^{-2} under saturating nitrate concentration. The response time and detection limit of sensor was 12s and 4.8µmol/l respectively. Nitrate biosensor in which platinum electrode modified a cellulose acetate membrane or with poly 1, 8-diaminonaphthalene film was assembled [4] and used for rapid amperometric detection of nitrate and nitrite in water by batch and flow injection analysis. Parameters such as permeability of electropolymerised films to nitrate, nitrites interference effects and recovery studies of these kinds of sensors have been optimized to demonstrate analytical suitability of proposed method.A biosensor in which enzyme catalyzed reduction of nitrate was studied [10] utilizing Aspergillus niger nitrate reductase (NaR) and phenosafranin in solution as enzyme regenerator electrode. The analytical parameters for enzyme modified electrode in presence of phenosafranin for determination of nitrate content in water was analyzed. Kinetic analysis of catalytic currents obtained in the presence of various amounts of nitrate has been used to identify and characterize the rate limiting steps governing the functioning of such an assembly.

Nitrate monitoring biosensor by immobilizing NaR (derived from yeast) on a glassy carbon or screen printed carbon paste electrode was prepared [21] using a polymer (polyvinyl alcohol) by entrapment method. Sensor could directly determine the nitrate in unpurged aquous solution with the aid of an appropriate oxygen scavenger nitrate reduction deriven by NaR and electron transfer mediator (methyl viologen) at 0.85 exhibited no oxygen interference in a sulfite added solution.Fabrication of a amperometric biosensor for specific determination of nitrate in water and meat samples by using nitrate reductase [8]. This biosensor was based on detection of oxidation peak current of redox mediator, methyl viologen, related to nitrate concentration. Dynamic range attained with this method was established as (5.0-90.0x 10(-9) M) for nitrate concentration with a

10 s response time, limit of detection as 2.2x10(-9) M and quantification as 5.79 x 10(-9) M.A nitrate biosensors by immobilizing glutaraldehyde on screen plated electrode represent important goal in amperometric nitrate biosensor development[2].Sensor could determine nitrate concentration in drinking water with the aid of azure B and methyl viologen electron transfer mediator .Detection limit found to be 0.5µm.An amperometric nitrate biosensor with an integrated permselective layer for exclusion of inorganic anions and cation interferences has been accomplished by formation of inner PPy(polypyrolle)-NaR-NADH layer of the biosensor by galvanostatic polymerization of pyrrole (Py) in presence of nitrate reductase (NaR) and nicotinamide adenine dinucleotide (NADH), followed by formation of the outer permselective poly-ortho-phenylenediamine (P-o-PDA) layer by potentiodynamic polymerization of ortho-phenylenediamine(o-PDA)[1]. The exclusion efficiency (Eeff) of the outer layer in rejecting inorganic cation and anion interferences was evaluated by a new proposed relationship. Further improvement in the exclusion efficiency for cations was accomplished by combining the use of the outer layer with the addition of 1mM EDTA into the measurement solution. The addition of EDTA improved the Eeff achieved for cation rejection by 10-40% to give net Eeff of 89-94%.the inclusion of the outer layer also aided the retention of NaR and NADH in the inner PPy-NaR-NADH layer and hence, enabled improved amperometric detection of nitrate, achieving a detection limit of 0.20µM and a linear concentration range of 10-500µM with a 3.4% rsd (n=10). After this,a amperometric nitrate biosensor based on polypyrolle(PPy/carbon nanotubes (CNTs) film has been constructed[9]**.**Nitrate reductase (NR) was both entrapped into the growing PPy film and chemically immobilized via the carboxyl groups of CNTs to the CNT/PPy film electrode.the optimum amperometric response for nitrate was obtained in 0.1M phosphate buffer solution(PBS), pH 7.5 including 0.1M lithium chloride and 7 mM potassium ferricynide with an applied potential of 0.13V (vs. Ag/AgCl,3M NaCl). Sensivity was found to be 300 nA/mM in a linear range of 0.44-1.45mM with a regression coefficient of 0.97. The biosensor response showed a higher linear range in comparison to standard nitrate analysis methods which were tested in this study and NADH based nitrate biosensors. A minimum detectable concentration of 0.17mM (S/N=3) with a relative standard deviation (RSD) of 5.4% (n=7) was obtained. Phenol and glucose inhibit the electrochemical reaction strictly at a concentration of 1µg/L and 20 mg/L,respectively. The biosensor response retained 70% of its initial response over 10 day usage period when used everyday.

Table-1: Nitrate biosensors from abroad have been summarized in table given below

Country	Biosensor type	Working electrode	Immobilization support	Detection limit	Disadvantages	Reference
France	Amperometric	-	N-substituted viologen polypyrolle film	4x 10^{-7}M	Sensitivity and stability of	CosnierS. and innocent

					biosensor was not good .show activity of leaching.	C.(1994)
Italy	Amperometric	Glassy carbon electrode	1-methyl-3-(pyrolle-1trimethyl)pyridinium	5.4µM	Solubilized form of nitrate reductase and electrocatalyst was slowed poor storage and determination conditions.	MorettoLMet .al (1998)
Germany	Amperometric	Graphite electrode	-	4.8µM/l	Co-immobilization of NaR and different mediators leads to decrease in kinetics of NaR and increase in response time.	Kirstein D. and Kirstein L.(1999)
Argentina	Amperometric	Gold electode	Polyvinyl/pyridinium/ /saffranin(self assembled layers)	-	The self assembled structure was leading to mass increment after the absorption of either polycation or enzyme .Due to this detection limit and sensitivity of sensor was decreased.	Ferreyra NF. et.al (2003)
Italy	Amperometric	Screen printed electrode	glutaraldehyde	0.5µM	Sensitivity was found low	D.albenese et.al(2010)
Australia	Amperometric	-	PPy(polypyrolle)	0.20µM	Leaching of enzyme can occur.	Manzar sohil (2011)
Australia	Amperometric	graphite	polypyrolle(PPy/carbon nanotubes (CNTs) film	0.17µM	Phenol inhibit the activity of enzyme.	Can,F.et al,(2012)

3. FUTURE PERSPECTIVES

Nitrate biosensors are currently experiencing a wealth of future developments in determination of nitrate level in drinking water. Now a days nitrate is becoming the most hazardous contaminant in drinking water and requires its determination by superior, efficient and cost effective method that is nitrate biosensor. Due to combination of advantages of electrochemical techniques with high nitrate specificity of nitrate reductase, quick response time and ease of procedure for nitrate determination,

it is promising research direction in fabrication of the most sensitive nitrate biosensors. The improvement in sensitivity and performance of nitrate biosensor requiring the development of novel biosensor. However practical utility of NaR-based biosensors have been increasing for nitrate monitoring because of their unique structural and electrochemical properties. Researches in the field of immobilization techniques, immobilization support and redox mediator have been creating the interest in the development of nitrate biosensor. Nitrate biosensors without interference of oxygen are employing now a day. Due to regenerator of NaR and high stability, nitrate biosensors have been used in nitrate determination in every type of sample. However, systematic studies of effect of hazardous nitrate level and nitrate determination by suitable method are still lacking, therefore future work must concentrate on addressing these biosensors.

4. CONCLUSION

Nitrate biosensors continue to attract enormous interest in determination of nitrate in water samples, food and biological fluids due to excellent electrochemical properties, sensitivity, cost effective and easy method of nitrate determination. These biosensors have electrochemical properties that are equal or superior to most of other methods or sensors. As a result of regeneration of NaR, nitrate biosensor is employing for long period of time for nitrate determination. In particular, their high aspect ratios allow nitrate biosensor to be improving their methodology during nitrate determination in water samples, food stuffs and biological samples. Moreover, chemical modifications of nitrate biosensor have been proved to be an effective way to impart selectivity to resulting biosensor, which could be exploited for the highly sensitive determination of nitrate with nitrate biosensor.

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