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Article

Nitrate Ion Selective Electrode Based on Ion Imprinted Poly(*N*-methylpyrrole)

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Abstract: A poly(*N*-methylpyrrole) based ion selective electrode (ISE) has been prepared by electro-polymerization of *N*-methylpyrrole using potassium nitrate as the supporting electrolyte. Electrochemical and chemical variables were used to optimize the potentiometric response of the electrodes and to maximize the selectivity for nitrate over potential interferences. The selectivity, longevity and stability of the ion-imprinted polymer give this electrode advantages over traditional nitrate ISEs. The best prototype electrode exhibits a linear potential response to nitrate ion within the concentration range of 5.0×10^{-6} to 0.1 M nitrate with a near Nernstian slope of −56.3 mV per decade (R^2 = 0.9998) and a strong preference for the nitrate ion over other anions. The selectivity coefficients of the electrode were evaluated by the fixed interference method. The use of *N*-methylpyrrole has advantages over pyrrole in terms of selectivity and pH insensitivity.

Keywords: ion selective electrode; conductive polymer; *N*-methylpyrrole

1. Introduction

Nitrate is an important ingredient in fertilizers. However, many species are susceptible to nitrate poisoning. The Environmental Protection Agency (EPA) has set the Maximum Contaminant Level (MCL) of nitrate as nitrogen at 10 mg/L and for nitrite 1 mg/L for the safety of drinking water [1]. Nitrate can be reduced to nitrite and ammonia, both more toxic than nitrate. The flora in the digestive tract of ruminants produces a significant amount of nitrite. This may result in anoxia since nitrite can oxidize the ferrous ion in hemoglobin to produce stable methemoglobin that is unable to transport oxygen. Methemoglobinemia is also known as “blue baby syndrome.” Nitrate contamination in ground water can result in such a large decrease in oxygen carrying capacity of hemoglobin and in babies it may lead to death. Ingested nitrate gets into saliva. The oral flora reduces some of the nitrate to nitrite. The nitrite that is swallowed has the potential of creating nitrosamines in the stomach that could lead to stomach cancer [2]. The bioavailability of ingested nitrate in vegetables has been determined to be 100% [3].

Since nitrate ion is an important analyte, a significant amount of effort has been expended to develop ionophores for nitrate. Nitrate is well known for being a weak coordinator requiring a complexation site of a specific size, shape and charge. Many approaches have been tried to prepare nitrate ionophores [4–6]. Most ionophores for nitrate are employed in conventional membrane ion selective electrodes. Conductive polymer-based solid-state ion selective electrodes are supplanting traditional membrane ion selective electrodes because of the advantage of not requiring a fill solution and because of their more rugged, easily miniaturized construction [7,8].

Kim et al. [9], Sun and Fitch [10] and Hutchins and Bachas [11] prepared ion selective electrodes for nitrate ion by electro-polymerization of pyrrole using a sodium nitrate supporting electrolyte.

Kim's electrode prepared by cyclic voltammetry exhibited a limited linear range, some selectivity and pronounced pH interference. Sun and Fitch prepared electrodes with a larger linear range, better selectivity and no pH dependence between 3 and 7. Hutchins and Bachas presented a very comprehensive study of the properties of the electrodes prepared by varying many of the parameters for preparation. Their optimized electrode was found to have better selectivity and sensitivity than conventional ion selective electrodes (ISEs) for nitrate or either of the two studies above. They did discover that the electrode also responded to changes in pH, requiring an ionic strength adjustment buffer.

N-methyl pyrrole was chosen as an alternative to pyrrole for two reasons: first, the methyl group on nitrogen should impede protonation in acidic media. This should impart less sensitivity to changes in solution pH. Second, *N*-methylpyrrole has been shown to electro-polymerize with a higher degree of branching and crosslinking [12]. Crosslinking is an important aspect of ionic imprinting. Higher levels of crosslinking are expected to improve polymer selectivity. It should be possible to prepare improved nitrate ISEs using *N*-methylpyrrole. Larraz, et al. [13] studied the influence of pH on the electro-synthesis of nitrate doped poly(*N*-methylpyrrole) films. The paper presented spectroscopic evidence that the polymer is protonated at low pH at the carbon alpha to the nitrogen. The rate of polymerization was faster at low pH and at pH of 1.0 the adhesion of the film was lessened. Later papers by some of the same authors decided that the protons were not bound but free [14]. Still, it was thought that the properties of ion-imprinted polymers prepared at differing pH values might show differing sensitivities and selectivities, since the spectral differences could be ascribed to structural differences in the polymer films.

2. Materials and Methods

Reagents. The *N*-methylpyrrole monomer, potassium salts of acetate, formate, nitrate, perchlorate, phosphate and sulfate were obtained from Sigma-Aldrich (Milwaukee, WI, USA). The remainder of the potassium salts was obtained from the following sources: chloride, bromide and iodide from Fisher Scientific (Cincinnati, OH, USA) and thiocyanate from Mallinckrodt Chemical Works (St. Louis, MO, USA). Aluminum oxide for chromatography was obtained from Fluka (Steiheim, Switzerland). The aqueous solutions were prepared using deionized (Milli-Q water purification system; Millipore, Bedford, MA, USA) reverse osmosis water.

Electrode preparations. The electrodes were purchased from BASi (West Lafayette, IN, USA). A conventional one compartment three-electrode electrochemical cell was used with a glassy carbon (GC) disk working electrode (area 0.07 cm^2), a platinum wire auxiliary electrode, and a silver chloride (3 M KCl) reference electrode. Prior to polymerization, the GC working electrode was polished with 0.3 μm alumina, rinsed with water, and cleaned ultrasonically. When the electrodes were exposed to organic solvent modifiers, the electrodes were heated to 50° C in vacuum for thirty minutes. *N*-methylpyrrole was deposited on the GC working electrode by galvanostatic electrochemical polymerization from a de-aerated aqueous solution containing 0.10 M *N*-methylpyrrole and 0.1M KNO_3 as the supporting electrolyte. *N*-methylpyrrole was purified immediately prior to use, by using an alumina column.

Potentiometric Measurements. All measurements were made with an eDAQ e-corder equipped with the eDAQ potentiostat and quad pH/millivolt amplifier (Denistone East, Sydney, NSW, Australia).

3. Results

Previous work with pyrrole established that optimal films were prepared using a constant current as opposed to a constant voltage [11]. Our tests agreed with this assertion, as we too found that a constant current electrode performed better than ones prepared with a constant voltage. Thus, a series of electrodes were prepared using a constant current for thirty minutes. The films were grown on 3.0 mm diameter GC electrodes. It was originally thought that an organic modifier was needed to obtain dissolution of the *N*-methylpyrrole, so the solutions were made using a 50:50 mixture of acetonitrile and water. (It was later discovered that *N*-methylpyrrole would dissolve to the extent required in deionized water.) The current values ranged from 10 µA to 120 µA. This resulted in films of increasing thickness. The electrodes were evaluated using serial dilutions of potassium nitrate in deionized water. Several trends were observed: the electrodes were slow to stabilize. As the films grew thicker, the slope of the calibration curve improved to a near Nernstian response. However, as the films grew thicker the response at low concentration decreased. At higher amounts of current, approaching 100 µA, the films buckled or lost adhesion with an abrupt and extensive change in morphology. It was observed that the films were damaged when higher currents resulted in a final potential in excess of 1.0 V.

Electrodes that exhibited good response to nitrate were tested with interferences. Polymer film anion electrodes have a natural propensity to bind lipophilic anions. The binding sequence is called the Hofmeister series [15], which gives rise to the following selectivity order: $\mathbf{H_2PO_4{}^- > C1^- > Br^- > NO_3{}^- > I^- > C1O_4{}^- > SCN^-}$. Initially, two anions were selected to determine selectivity, I^- and Cl^-. It was found that selectivity for these two anions was improved slightly with increasing film thickness. However, it was observed that the best selectivity, measured for an electrode made using 80 microamps of current, degraded over the period of a week. In general, the selectivity, in terms of selectivity coefficients as measured using separate solutions were on the order of 10^{-2} for I^- and 10^{-3} for Cl^-. The tests were repeated using a solution that was a 50:50 mixture of acetonitrile and water. The responses were more rapid and the lower concentration responses were restored.

The initial study revealed several issues: the electrodes respond rapidly as long as they are tested in a solution that has the same solvent composition as the growth solution. Dilutions of the ions in de-ionized water responded slowly and erratically, while dilutions in the 50:50 mixture of acetonitrile and water responded rapidly. Other organic modifiers that were less expensive and more environmentally benign, such as ethanol and surfactants were also tested. Ethanol made films that appeared more hydrophobic and inhibited the nitrate response versus the iodide response. There was the expectation that a surfactant could also enhance response, since it would plasticize the membrane and act as an auxiliary ionophore. The nonionic surfactant used, Polysorbate 80, made films that behaved in a manner similar to ethanol solutions. Also, reusing glassy carbon electrodes that have been exposed to organic solvents exhibited a diminishment of function, likely due to absorbance of organic solvents. This was remedied by heating the electrodes in vacuum. When it was determined that *N*-methylpyrrole would dissolve in de-ionized water given time and sonication, the use of additional miscibility agents was eliminated.

All further work employed aqueous solutions. The response of a typical electrode is seen in Figure 1. The electrodes responded rapidly, within seconds, and then maintained a constant potential. The use of the eDaq recorder allowed observation of the potential for any time interval needed. Previous reports have suggested that electro-active polymer films may be photo unstable. Thus, the electrodes were stored in a 10^{-3} M KNO_3 solution residing in brown glass vials. The electrodes were observed to improve in terms of sensitivity with conditioning, Figure 2. Most of the improvement was in a few days but small improvements could be observed for up to several weeks. This may be due to the greater amount of crosslinking with *N*-methylpyrrole. It is expected that conditioning will allow for the release of unreacted monomers or short oligomers trapped in the film during preparation. This removal would reduce nonspecific binding sites and thus improve the selectivity and sensitivity of the electrodes. It was noticed that using fresh conditioning solutions daily shortened the time needed to condition the electrodes. There was no loss in function observed after storage for six months.

Another way to approach the removal of oligomer and unreacted monomers is by undoping the polymer with an applied voltage. A fresh electrode was prepared using 70 µA of current for 30 min. The electrode was undoped by applying -300 mV for 30 min following the current graphically. The current had essentially fallen to zero leaving the film undoped. The electrode was conditioned overnight and tested the next day. The electrode exhibited a near-Nernstian slope of 54.7 mV/decade and a rapid response. This is similar to the response that required two weeks for an electrode that was not undoped. The difference from a long-term conditioned electrode was that the low concentration response had not developed. The low-end response did improve slightly with additional conditioning. However, the undoped electrode did not have the degree of selectivity of the electrodes that were not undoped, Figure 3. This suggests that the undoping process changed the films structure, possibly collapsing some of the pores and distorting the imprinted sites.

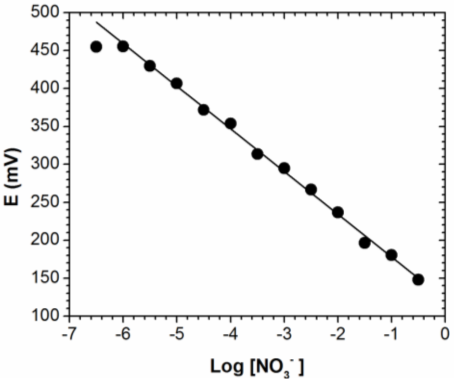

Figure 1. Potentiometric response of an optimized poly(*N*-methylpyrrole)-based film ion selective electrode (ISE). Data are fitted to a logarithmic curve of equation E = −56.33 log[NO_3^-] + 121.4, R^2 = 0.9974, for data points from [NO_3^-] = 0.5 to 10^{-6} M.

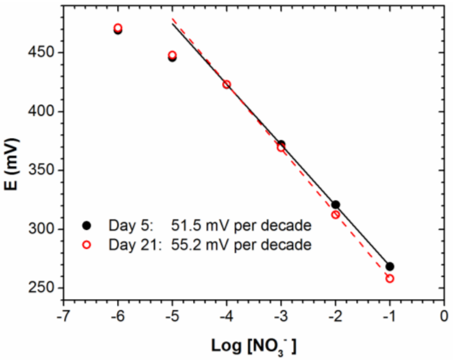

Figure 2. Effect of conditioning of poly(*N*-methylpyrrole)-based ISE's on response to nitrate anion in aqueous solution. Electrodes were soaked in 10^{-3} M KNO_3 solution immediately after film growth on day 1 then tested on day indicated. Fit is to a logarithmic curve, with equation E = m log[NO_3^-] + b, using data points from [NO_3^-] = 10^{-1} to 10^{-4} M. For day 5 results, E = −51.5 log[NO_3^-] + 217.1, R^2 = 0.9999. For day 21 results, E = −55.2 log[NO_3^-] + 202.8, R^2 = 0.9998.

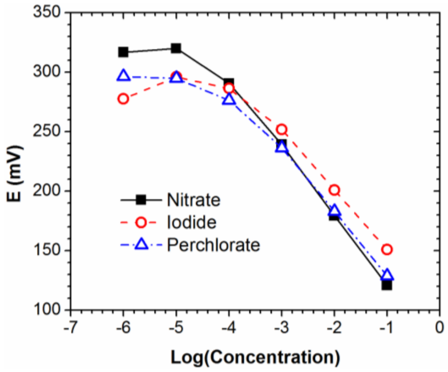

Figure 3. Response of a conductive polymer film ISE for nitrate that was undoped after film growth. Response to nitrate and interferent anions iodide and perchlorate shows little selectivity for nitrate against the interferents.

The response of poly(*N*-methylpyrrole) imprinted with nitrate ions was evaluated with changes in pH. The results are shown in Figure 4. The results of this study is compared to those employing poly(pyrrole) imprinted with nitrate ions [11]. As is seen in Figure 4 there is very little pH dependence for this electrode. The immunity to pH changes imparts additional flexibility to this ISE.

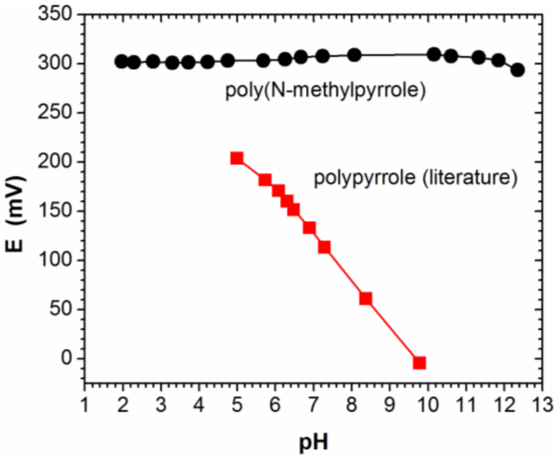

Figure 4. Effect of pH on response of nitrate ISE's using polypyrrole and poly(*N*-methylpyrrole) films. Poly(*N*-methylpyrrole) is relatively insensitive to pH. Polypyrrole data are taken from [11].

There are reports relating differences in poly(*N*-methylpyrrole) films prepared at differing pH values [13,14]. One report states that some of the polymer was protonated at the α carbon when prepared or conditioned at low pH. This implies that the complexing ability of films prepared at different pH values may vary. Electrodes were prepared at three different pH values and their responses tested. This was done to see how poly(*N*-methylpyrrole) protonated at the α carbon would affect the response of the electrodes. While there seems to be some effect, the effect is not dramatic. Table 1 shows the selectivity coefficients for our electrodes and those from [11]. The selectivity coefficients were obtained using the fixed interference method for the purpose of direct comparison. It is apparent that the *N*-methylpyrrole-based electrodes are more selective for nitrate than pyrrole-based electrodes. This supports the claim that *N*-methylpyrrole undergoes a greater degree of crosslinking. However, while there are some small differences in selectivity at differing pH, there doesn't appear to be a usable trend. It does appear that raising or lowering the pH of fabrication improves selectivity against hard divalent anions.

Table 1. Selectivity coefficients for nitrate ion selective electrodes.

Interfering Ion	**Selectivity Coefficients at Specified pH**			**Comparison to Literature Values, from [11]**
	pH 6.14	**pH 3**	**pH 9**	
Acetate	5.01×10^{-4}	3.16×10^{-4}	2.00×10^{-4}	NA
Bromide	7.94×10^{-4}	7.94×10^{-4}	6.31×10^{-4}	7.6×10^{-2}
Phosphate	2.82×10^{-3}	3.16×10^{-4}	3.16×10^{-4}	9.0×10^{-4}
Sulfate	3.16×10^{-4}	1.99×10^{-5}	2.0×10^{-5}	6.0×10^{-4}
Iodide	3.98×10^{-3}	2.51×10^{-3}	1.26×10^{-3}	5.1×10^{-2}
Formate	7.94×10^{-4}	3.98×10^{-4}	3.98×10^{-4}	NA
Thiocyanate	6.31×10^{-3}	1.00×10^{-2}	5.01×10^{-3}	3.6×10^{-1}
Perchlorate	5.01×10^{-3}	5.62×10^{-3}	5.01×10^{-3}	5.7×10^{-2}

4. Discussion

Fourier Transform Infrared Spectroscopy (FT-IR) was used to examine the films prepared at differing pH values. The spectra were obtained using a diamond anvil ATR. The report cited above also used FT-IR to compare the films at differing pH values [13]. Those films were prepared at fixed potential of 0.8 V versus a saturated calomel electrode (SCE) resulting in considerably higher current densities than were used in the current study. The IR spectra of films grown at pH 9 and pH 3 are given in Figure 5. There are differences in the spectra for bands around 1300 cm^{-1} and 650 cm^{-1}. These bands have been assigned to the polymer back bone, [13] suggesting small changes in the polymer conformations. There is a marked difference in the spectra obtained on films prepared in this study as compared to this previous report. All of the spectra from the previous study showed a carbonyl band at about 1700 cm^{-1}. Of the spectra obtained in this study only two had a peak near this position. One was for a benzoate electrode that required significantly more voltage to polymerize that had a weak band at 1736 cm^{-1}. The other spectrum was obtained for a film prepared using sulfate as the supporting electrolyte with a band was at 1684 cm^{-1}. It was observed that the level of over oxidation caused by the higher potentials and currents usually result in films unsuitable as sensors.

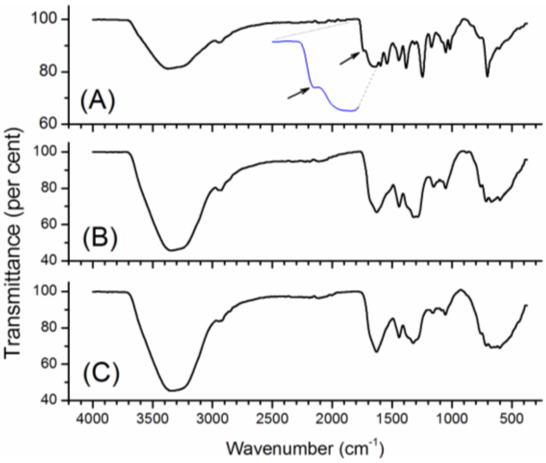

Figure 5. IR spectra of films produced under conditions of varying pH and dopant anion. (**A**) Benzoate. Inset shows the region from 1850 to 1620 cm^{-1}, and arrow points to feature in the benzoate-doped electrode spectrum at ca. 1736 cm^{-1}, assigned to a carbonyl stretch mode; (**B**) Nitrate, pH 3; (**C**) Nitrate, pH 9.

Scanning electron micrographs show that the morphology of the films is similar to others reported in the literature. An example is given as Figure 6.

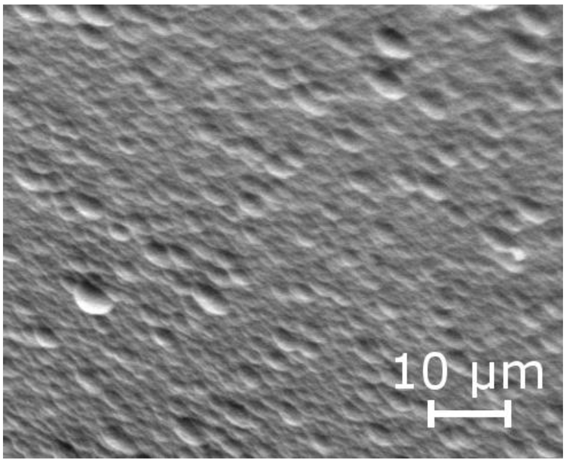

Figure 6. Scanning electron micrograph of a typical poly(*N*-methylpyrrole) film.

In order to verify that imprinting induces selectivity, control experiments were performed. Electrodes were prepared using the optimal conditions previously established for nitrate while substituting chloride and perchlorate as supporting electrolytes. The electrodes were conditioned and then tested for response to the imprinting ion, nitrate and other interferences. The chloride and perchlorate electrodes responded to changes in concentration but did not show a preference for the imprint ion over nitrate. This is contrary to reports for pyrrole electrodes prepared for these anions [16,17].

Additional Analytes. Due to the success with nitrate it was considered reasonable to try to prepare electrodes for other anions. Electrodes were prepared using the optimal growth conditions found for nitrate ions for additional anions, both univalent and divalent: benzoate, chloride, dicyanoargentate, perchlorate, picrate, carbonate, sulfate and oxalate. The results were that dicyanoargentate and picrate electrodes functioned polymerized with voltages similar to nitrate and performed with good sensitivity and selectivity. The chloride and perchlorate electrodes polymerized with voltages similar to nitrate and performed with adequate sensitivity but poor selectivity. The electrodes prepared for carbonate and oxalate did not form films. The electrodes prepared for sulfate formed films at reasonable voltages but the electrodes did not respond in a consistent manner. The electrode prepared for benzoate was both selective and sensitive, but formed at significantly higher voltage. From these experiments it is possible to make some generalizations. It appears that univalent planar anions are good candidates for imprinting with poly(*N*-methylpyrrole). Other univalent non-planar anions can be used to make electrodes that respond to anion concentration but these dopants do not form films that are selective for the imprint. Divalent anions are unsuitable for preparing electrodes. This problem may have an explanation. A paper examining the effect of sulfate supporting electrolyte on the polymerization of *N*-methyl poly(pyrrole) found that the films grown were heavily over oxidized [18]. The paper suggests that a divalent anion could stabilize a divalent cation that would more easily hydrolyze and then oxidize to two carbonyl groups. This is consistent with the IR spectra that show a strong carbonyl band for films grown using sulfate as the supporting electrolyte. Additional experiments will be performed to obtain detailed data on the responses of the other selective electrodes.

5. Conclusions

Poly(*N*-methylpyrrole) can be ion imprinted by electro-polymerization of *N*-methylpyrrole using potassium nitrate as the supporting electrolyte. The selectivity of the ISE produced by this process exceeds that of other solid-state ISEs for nitrate. Changes in growth parameters such as current, voltage and pH were investigated. The selectivity, longevity and stability of the ion-imprinted polymer give this electrode advantages over traditional nitrate ISEs. The best prototype electrode exhibited a linear potential response to nitrate ion within the concentration range of 5.0×10^{-6} to 0.1 M nitrate with a near Nernstian slope of -56.3 mV per decade ($R^2 = 0.9998$) and a strong preference for the nitrate ion over other anions. The use of *N*-methylpyrrole has advantages over pyrrole in terms of selectivity and pH insensitivity. The pH insensitivity of the poly(*N*-methylpyrrole) matrix and the more extensive crosslinking suggests that this polymer is a useful matrix for the preparation of other ion imprinted electrodes.

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Author Contributions: George M. Murray conceived and designed the experiments; Ellen M. Bomar and George S. Owens performed the experiments; George S. Owens analyzed the data; George S. Owens and George M. Murray wrote the paper.

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