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A COATED-WIRE ION-SELECTIVE ELECTRODE FOR IONIC CALCIUM MEASUREMENTS

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ABSTRACT

A coated-wire ion-selective electrode for measuring ionic calcium has been developed, in collaboration with Teknekron Sensor Development Corporation (TSDC), Menlo Park, CA. This coated wire electrode sensor makes use of advanced, ion-responsive polyvinyl chloride (PVC) membrane technology, whereby the electroactive agent is incorporated into a polymeric film. The technology greatly simplifies conventional ion-selective electrode measurement technology, and is envisioned to be used for real-time measurement of physiological and environmental ionic constituents, initially calcium. A primary target biomedical application is the real-time measurement of urinary and blood calcium changes during extended exposure to microgravity, during prolonged hospital or fracture immobilization, and for osteoporosis research. Potential Advanced Life Support applications include monitoring of calcium and other ions, heavy metals, and related parameters in closed-loop water processing and management systems. This technology provides a much simplified ionic calcium measurement capability, suitable for both automated *in-vitro*, *in-vivo*, and *in-situ* measurement applications, which should be of great interest to the medical, scientific, chemical, and space life sciences communities.

INTRODUCTION

Measurement of chemical and organic constituents is a critical requirement for many hospitalized patients. Parameters such as ionic calcium (Ca^{2+}), potassium (K^+), and blood gas constituents (pH, PO_2 , PCO_2) are of great interest for determining the clinical and physiological implications of illness and various therapies as well as long-term spaceflight missions. As part of NASA's planned Life Sciences and Advanced Life Support Space Technology Programs, we are developing minimally invasive technologies which can be used to measure important ionic constituents on Earth and during prolonged spaceflight. This technology can also be applied to monitor and control such necessities as water and air quality, and to effect closed-loop control on reclamation and management of those processes. The calcium CWE represents the initial demonstration of this technology.

Ion-selective electrodes are becoming increasingly important in measuring ion concentrations in solutions. The continuing study of and application of liquid and polymer membrane ion-selective electrodes (ISE) remains a strong and viable area of interest in several laboratories. The theory behind the operation of ion-exchange membranes has been investigated and developed on the basis of the concept of zero-current potential. Ion-selective electrodes have made great inroads into the testing of electrolytes and have replaced flame photometers for this use in many laboratories. Ion selective electrodes have found application in the control of industrial processes, water supplies and waste water. In addition, they are used extensively in biomedical applications. Of the liquid membrane-based electrodes incorporating ion-exchange materials, calcium-selective electrodes in particular continue to draw interest. Much of the work has been directed to the way in which electrodes are constructed. The desire to miniaturize, simplify and to produce cheaper ion-selective electrodes has engrossed a diverse group of research workers.

Presently no reliable sensors exist for undertaking real-time *in vitro*, *in vivo*, and *in-situ* measurements during space flight missions. The development of such microsensors will permit real time monitoring of astronaut health and especially, gradual degenerative changes such as bone mass loss, which occurs at or near zero gravity. The

development of a suitable calcium microsensor for medical and physiological measurements will not only have profound applications for NASA but also for the study of osteoporosis - a disorder which affects many postmenopausal women.

Advanced Life Support requirements for such planned mission scenarios as Lunar and Martian bases have placed stringent demands on sensors, and measurement and control technology for closed-loop physical/chemical water and air quality systems. The list of required and potentially required parameters include ionic constituents, heavy metals, detergents, microbes, organics and trace contaminants, and microbial constituents, such as bacteria and viruses. The Coated-Wire Calcium Electrode is but one possible method for measuring such constituents, but represents an important approach due to the simplicity of the configuration.

Conventional ion-selective electrodes

The conventional cell arrangement for potentiometric measurements using an ion-selective electrode or membrane electrode is shown in figure 1, below.

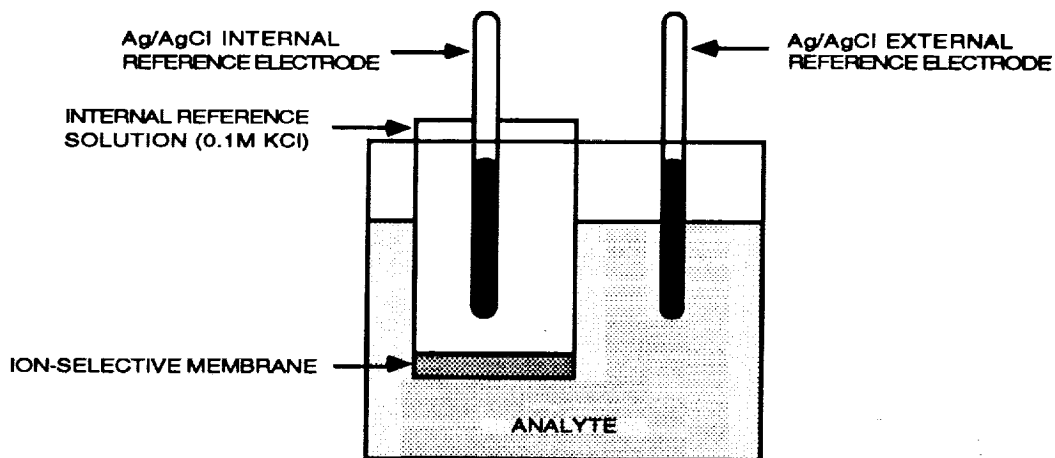


FIGURE 1: EXAMPLE OF A CONVENTIONAL ION-SELECTIVE ELECTRODE

In this configuration, the internal aqueous reference solution, which contains the ions necessary to maintain a constant potential at both the reference element and the inner surface of the ion-selective membrane is held at a constant composition. The potential difference across the cell is dependent on the composition of the sample solution, and the specific ion that will be detected is determined by the nature of the membrane.

Coated wire ion-selective electrodes

The coated wire electrode (CWE) uses components of conventional ion selective electrodes except that no internal aqueous filling solution is used (figure 2). Instead a conductor is directly coated with an ion-responsive membrane, usually polyvinyl chloride (PVC) based. Thus in a coated wire electrode, the electroactive agent is incorporated into a polymeric film. The conductor can be metallic or graphite-based and be of any convenient geometric shape (i.e. wire, disk, cylinder, thin film, etc.). When the ion-selective membrane comes into contact with an ionic solution an ion exchange reaction takes place resulting in a potential difference across the solution-membrane interface. In coated-wire electrodes the cell configuration is as follows:

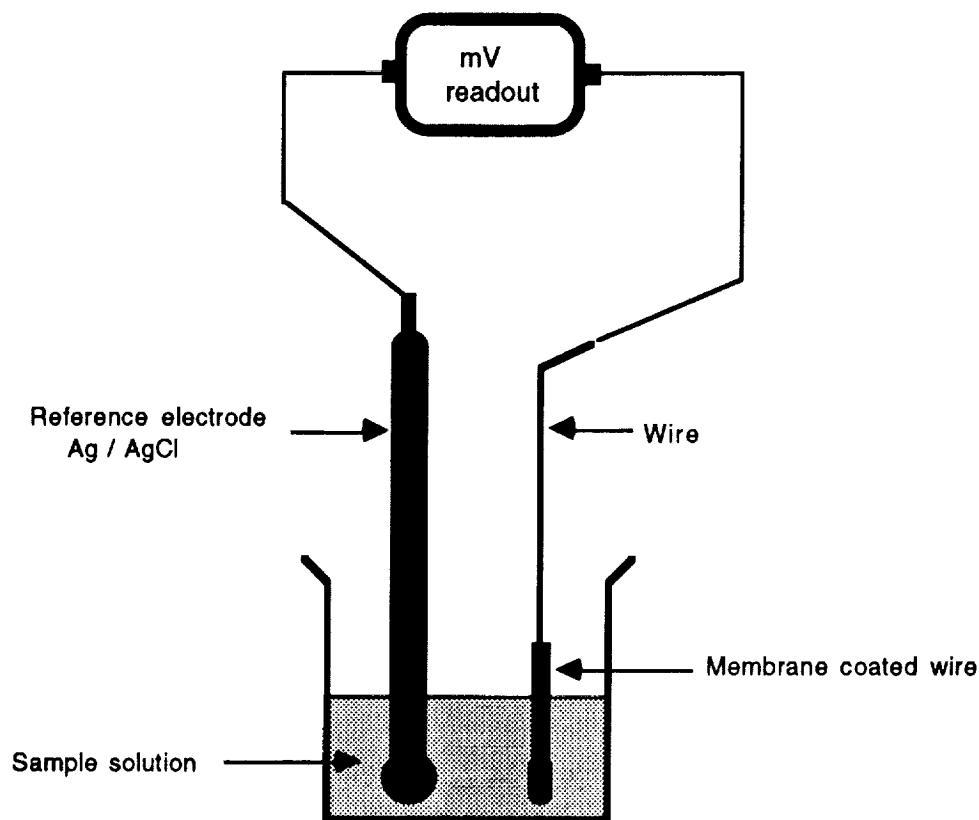


FIGURE 2: EXAMPLE OF A COATED WIRE ELECTRODE

EXPERIMENTAL METHODS

Initial Development Phase

The general procedure for making a Coated Wire Electrode is given below:

1. Preparation of the membrane solution

The membrane solution for a Ca^{2+} ion-selective electrode was prepared using an optimized formulation consisting of a Calcium ionophore, specific binding and reactive agents, and polyvinyl chloride.

2. Application of membrane to silver wire

A straight piece of silver wire 8 cm long, previously cleaned, was dip-coated several times by immersing it into the above membrane solution. Great care was exercised to prevent the occurrence of any pin-holes by ensuring that a relatively thick membrane was coated on to the wire as shown in figure 3 below:

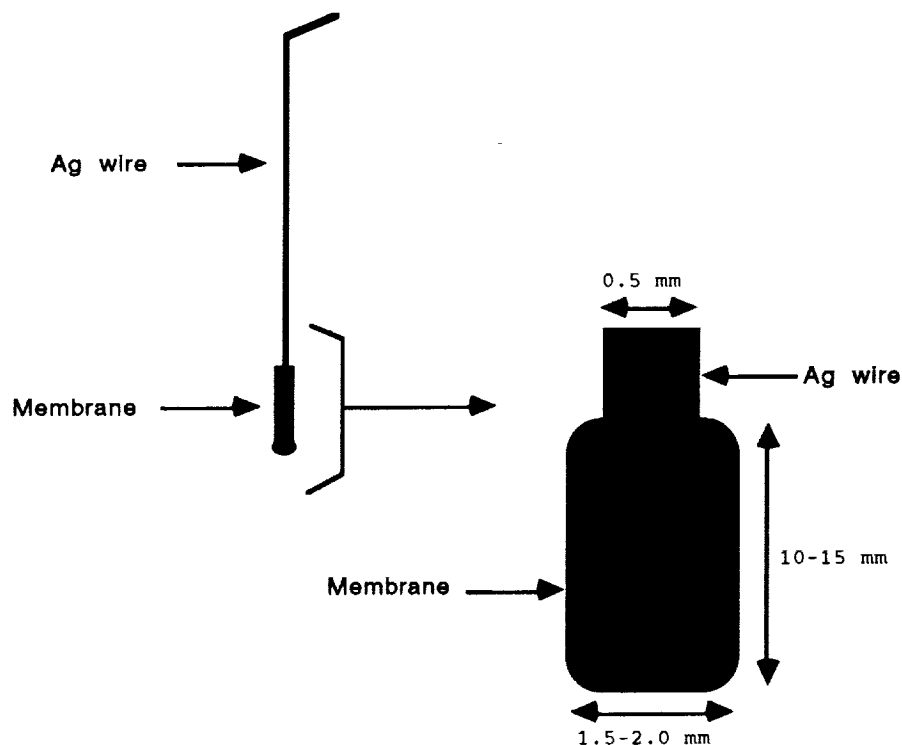


FIGURE 3: REPRESENTATION OF A CALCIUM ION SELECTIVE MICROELECTRODE

In applying a thick membrane coating, the main aim is to provide good adhesion to the metal surface and to obtain a film devoid of pin holes. It should be noted that although the 'dip-coating' procedure applies specifically to wires, it can also be adapted for other configurations such as plates and discs. If necessary the remaining exposed metal surface of the electrode can be insulated by tightly wrapping parafilm around it. After application of the membrane coat, the electrodes were allowed to dry for several hours or overnight before measurements were taken. In some instances the electrodes were stored dry for three weeks before taking measurements. Prior to taking any readings the electrodes were allowed to equilibrate in a dilute calcium chloride solution for about an hour. This precaution is usually only needed initially, since subsequent equilibration times are much shorter (a few minutes).

3. Initial testing of electrodes

All measurements and tests were conducted using the experimental setup illustrated in figure 4 below:

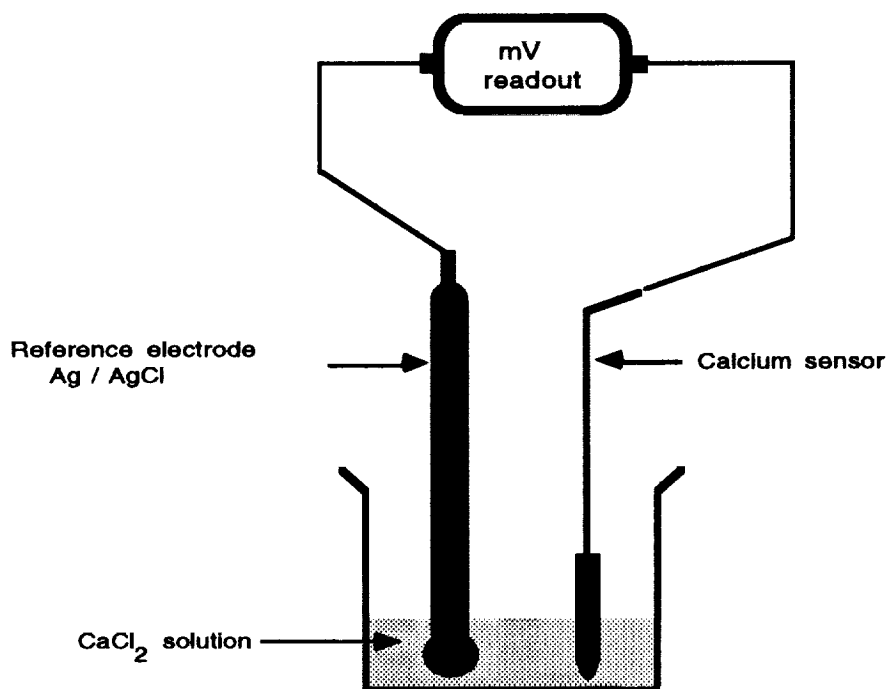


FIGURE 4: EXPERIMENTAL SETUP USED FOR TESTING CALCIUM WIRE ELECTRODES.

An external Ag/AgCl electrode was used as a reference electrode. Both the calcium ion selective electrode and the Ag /AgCl reference electrode were connected to an Orion pH / mV monitoring unit as shown above. For this initial evaluation, a set of standard CaCl₂ solutions ranging in concentration from 1 x 10⁻¹ - 1 x 10⁻⁵ M and containing 0.1 M KCl were prepared, lacking any chelating agents such as EDTA or EGTA, to determine the slope and response of the microelectrodes.

EXPERIMENTAL RESULTS

Under ideal conditions, the potential difference across the solution-membrane interface is given by the Nernst equation:

$$E = \frac{RT}{ZF} \ln a_{Ca^{2+}},$$

where *Z* and *a* are the charge and activity of the ion, *R* is the gas constant, *T* is the temperature and *F* is the Faraday constant. The theoretical sensitivity as predicted by the Nernst equation is approximately 58 mV/pH unit at ambient temperature for a monovalent cation and about 29 mV/pH unit for a divalent cation.

Several of the Ca²⁺ ion selective electrodes were tested using the experimental setup shown in figure 4. Typical response curves for four such electrodes are shown in figure 5 below:

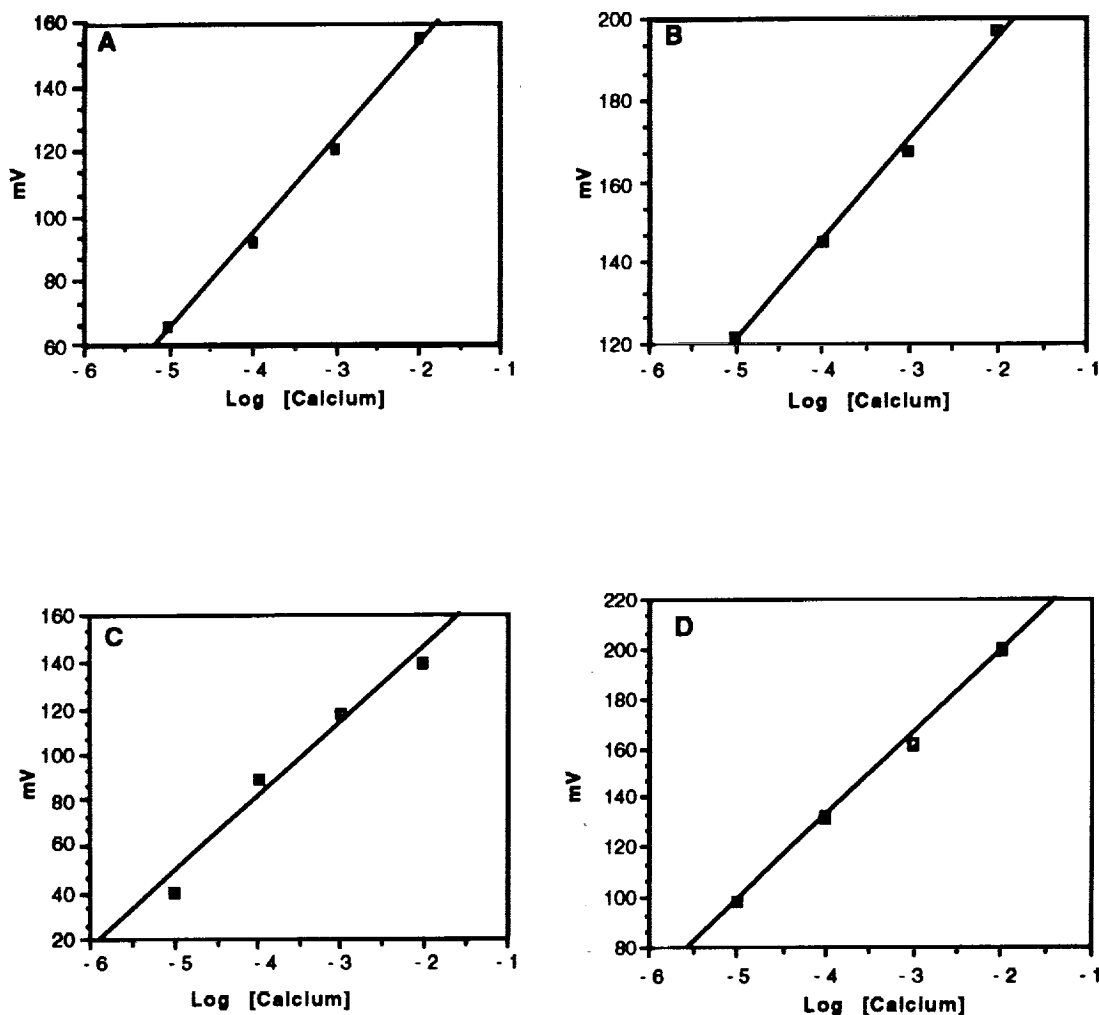


FIGURE 5: RESPONSE CURVES OF CALCIUM ION SELECTIVE MICROELECTRODES

The tests were conducted by measuring the responses progressively in increasing concentrations of CaCl_2 solutions. Although the initial potentials of the above electrodes in 1×10^{-5} M CaCl_2 solution varied, the average decade change between solutions is about 26 mV which compares favorably with the predicted theoretical value of a divalent cation according to the Nernst equation. When the electrodes were immersed in solution for 2 - 4 hours the electrode potentials displayed negligible drift, thus indicating that the Ca^{2+} ion selective microelectrodes have relatively stable potentials. However, it should be noted that, at this stage in the development, the construction of these electrodes is much of an art and therefore it is quite possible that microelectrodes made from the same batch could vary considerably in their properties.

Comparison of the Coated Wire Electrode with Reference Instrumentation

After having established the slope of the response of the coated wire sensors, we undertook a study to determine the compatibility of the Ca^{2+} selective membrane with the standard, commercially available Radiometer electrode assembly. An enlarged top view is presented in Figure 6.

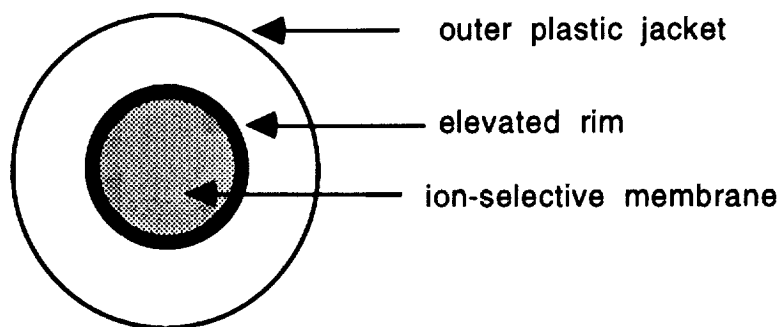


Figure 6: Top View of Coated Membrane

The original membrane on the Radiometer electrode was carefully removed and the surface cleaned with water and isopropanol. The calcium selective membrane was then immobilized onto the clean surface. A thick coating was applied to ensure a membrane devoid of pin holes. The immobilized membrane was then allowed to air-dry overnight. The electrode was reassembled as described by the manufacturer (Radiometer Copenhagen). We experienced great difficulty in removing air bubbles that were trapped in the jacket containing the electrolyte solution. The air bubbles prevent intimate contact between the membrane and the internal electrolyte solution. We believe the present design of the Radiometer electrode can be improved considerably to avoid these problems. To ensure reasonable contact between the internal electrolyte solution and the membrane, a short wick (made of cotton thread) was introduced. Two such electrodes were prepared and reassembled.

Both reassembled Radiometer electrodes containing the calcium ion selective membrane were then tested initially using standard CaCl_2 solutions (as shown in Figure 2) with the Radiometer Model ICA1 Ionized Calcium Analyzer. During initial, standalone testing, we observed a somewhat linear response to increasing CaCl_2 solutions. Due to excessive signal noise, it was not possible to obtain steady readings. However, when the electrodes were tested with the Radiometer Model ICA1 Ionized Calcium Analyzer, we obtained a very good response to various known concentrations of CaCl_2 solutions. The response of these electrodes compared very favorably with previous results obtained using the standard, commercial electrode system. For example, after calibration of these electrodes with the calibration solutions provided by the manufacturer the following results were obtained:

TSDC Electrode #1

| | | Measured | Expected |
|------------|---------------------------------------|-----------------|-----------------|
| Solution A | Ca^{2+} concentration mmol/l | 1.26 | 1.25 |
| Solution B | Ca^{2+} concentration mmol/l | 0.75 | 0.69 - 0.83 |
| Solution C | Ca^{2+} concentration mmol/l | 1.75 | 1.69 - 1.83 |

TSDC Electrode #2

| | | Measured | Expected |
|------------|---------------------------------------|-----------------|-----------------|
| Solution A | Ca^{2+} concentration mmol/l | 1.28 | 1.25 |
| Solution B | Ca^{2+} concentration mmol/l | 0.79 | 0.69 - 0.83 |
| Solution C | Ca^{2+} concentration mmol/l | 1.82 | 1.69 - 1.83 |

SUMMARY AND CONCLUSIONS

These results strongly indicate that the Calcium ion selective membrane is compatible with the commercial Radiometer electrodes. In addition, this membrane meets the requirements of sensitivity and selectivity to develop a Microcalcium Sensor for *in-situ* and *in vivo* measurements of ionic calcium. During the next phase of this project, we plan to evaluate the long-term stability of the ion selective membrane, and expand the technology demonstrations into more rigorous feasibility studies, using representative, physiological, clinical, and process control water stream samples. We intend to continue the development efforts to a usable prototype level, which can then be used in research, testbed, and operational spaceflight mission development activities. The Coated-Wire Electrode, when coupled with appropriate measurement instrumentation, has potential commercial application as a small, self-contained, portable instrument, capable of real or near real-time usage.