Potassium-selective potentiometric microelectrodes with a polypyrrole solid internal contact were fabricated by the application of a potassium-selective bis-crown ether ligand based, plasticized poly(vinyl chloride) (PVC) membrane to the surface of conducting polymer modified Pt, Au or C micro disk electrodes. The selectivity and sensitivity of the new type of potentiometric potassium microelectrode were found to be comparable with those of the conventional macro ion-selective electrodes and of the micropipet type microelectrodes based on the same ionophore. The ease of preparation and robustness are the main advantages of this new electrode design, which can replace the classical micropipet type microelectrodes in many applications. The microelectrodes showed good dynamic characteristics and were used successfully in a wall-jet cell incorporated flow injection analysis system and as a measuring tip in scanning electrochemical microscopy. The polypyrrole modified substrate electrodes can be made selective for different ions just by changing the ionophore in the plasticized PVC membrane, which can extend their use to a wide range of applications.

Keywords: Potentiometric microelectrode; potassium-selective electrode; polypyrrole; scanning electrochemical microscopy; flow injection analysis

The preparation and application of microelectrodes have attracted much interest in recent electroanalytical chemistry studies. This is part of a general trend in analytical chemistry towards miniaturization. The features of voltammetric microelectrodes have been investigated extensively during the last few years with the aim of improving the characteristics of electrochemical methods and performing studies under conditions not possible with conventional electrodes. A variety of electrode materials of different shapes and sizes have been reported, but the most commonly used electrode materials are platinum, gold and carbon fiber.

Potentiometric microelectrodes were fabricated decades earlier than voltammetric microelectrodes because of the need for life scientists to monitor ion activities in living organisms. The most commonly used potentiometric microelectrode design is the micropipet type, which can be prepared with an advantageously small tip size. Some of them have a tip diameter of a few tens of nanometers. However, such microelectrodes are delicate tools and working with them requires special skill and instrumentation. They suffer from a lack of robustness and mechanical resistance. These disadvantages can be avoided if the traditional micropipet configuration is replaced with an internal solid contact.

The replacement of the classical internal filling solution in potentiometric sensors is important because that design offers a number of advantages such as simplicity, lower cost, better mechanical flexibility and possibility of miniaturization. In ion-selective electrode research there have been many attempts to replace the conventionally employed internal filling solutions with solid contacts. In particular, the coated wire electrode attracted much attention. Coated wire electrodes were prepared simply by coating an ion-selective film directly on a metallic conductor. Although these electrodes have a simple design and good mechanical stability, they did not provide a stable and reproducible potential response. Buck6 pointed out that the problem with coated wire electrodes can be attributed to the fact that the interface between the substrate metal and the ion-selective membrane is blocked, i.e., thermodynamically ill-defined. Many efforts have been made over the years to solve the problem of the blocked interface between the substrate metal and the ion-selective membrane with ionic conductivity, e.g., by the dispersion of redox couples in the membrane phase and by the use of hydrogels or epoxy layers loaded with metal.10–12

The use of conducting electroactive polymers (CEPs) such as polypyrrole (PPy), polythiophene (PT) and polyaniline (PANI) as the active component of electrochemical sensors has attracted much attention in recent years. It was reported that polypyrrole and poly(3-octylthiophene) films can mediate charge between ionic (specific to ion-selective membranes) and electronic (specific to metal substrates) conductive layers. Based on this principle, all-solid-state, conventional size sodium-selective electrodes have been fabricated and their analytical parameters have been determined. The sodium-selective electrodes showed no redox sensitivity and exhibited good potential stability.

Polypyrrole has already been used in miniaturized analytical sensors such as microelectrodes and microelectrode arrays, but to the best of our knowledge no polypyrrole based solid contact potentiometric microelectrodes have yet been prepared. We describe here the preparation and analytical properties of novel robust and mechanically flexible, polypyrrole interfaced potassium-selective microelectrodes with a solid contact. For this study, a potassium-selective ionophore, BME-44, incorporated in a plasticized poly(vinyl chloride) (PVC) membrane was used on different polypyrrole modified substrates. These new potentiometric microelectrodes were tested as measuring tips in scanning electrochemical microscopy (SECM) to monitor the voltammetrically inaccessible potassium ion concentration profiles.

**Experimental**

**Chemicals**

Pyrrrole, obtained from Ventrion (Karlsruhe, Germany), was distilled prior to use and kept at low temperature, protected from light. All the other chemicals of analytical-reagent grade, such as lithium acetate (LiOAc) (Fluka, Buchs, Switzerland), ultra low gelling temperature agarose (SeaPrep, FMC, Rockland, ME, USA), KCl, NaCl, KNO3 (Reanal, Budapest, Hungary) and
Preparation of disk-in-glass microelectrodes.

Each step is very important in order to obtain a micro ion-selective membrane. A potassium-selective membrane was placed over the polypyrrole deposited by electropolymerization on the disk and finally (3) a gold wire and carbon fibers of different sizes were obtained from Goodfellow (Cambridge, UK). De-ionized water was used for the preparation of all solutions.

**Instrumentation**

A BAS 100B electrochemical workstation (Bioanalytical Systems, West Lafayette, IN, USA) with a BAS PA-1 pre-amplifier was used in the electrochemical polymerization and the cyclic voltammetric experiments. During the measurements the electrochemical cell was placed in a Faraday cage (BAS Cell Stand C2). The electrochemical polymerization of pyrrole and the study of the polypyrrole film were performed in a three-electrode cell arrangement. The working electrode was either a platinum (diameter 5 or 25 μm), a carbon fiber (diameter 8 or 30 μm) or a gold (diameter 100 or 300 μm) microelectrode. The reference electrode was an Ag/AgCl electrode and a Pt wire was used as the auxiliary electrode.

For the potentiometric characterization of the microelectrodes, an OP-208/1 precision digital pH-meter (Radelkis, Budapest, Hungary) was used. The reference electrode in this case was a saturated calomel electrode (SCE) with a 0.1 M LiOAc salt bridge.

The flow injection analysis set-up included a peristaltic pump (Ismatec, Glattbrugg-Zürich, Switzerland) and a four-way injector (Rheodyne, Cotati, CA, USA).

The SECM images were recorded with a laboratory-made scanning electrochemical microscope. The SECM set-up has been described in detail elsewhere. The positioning device of the microscope is based on three high resolution (75 nm) and 1340 nm) precision stepper motor driven translation stages (Newport, Irvine, CA, USA) with a BAS 100B electrochemical workstation (Bioanalytical Systems, West Lafayette, IN, USA) with a BAS PA-1 pre-amplifier. The positioning system was controlled by a Pentium-75 computer through a PC-Lab 812 interface.

An Axiolab A (Zeiss, Oberkochen, Germany) optical microscope was used in order to inspect the microelectrodes after each preparation step. The micropipettes employed as the target in SECM measurements were prepared using a vertical pipette puller (Model 720, KOPf, Tujunga, CA, USA).

**Microelectrode preparation**

In the preparation of the microelectrodes, three steps can be distinguished: (1) first the so-called disk-in-glass microelectrodes were prepared, then (2) the polypyrrole was deposited by electropolymerization on the disk and finally (3) a potassium-selective membrane was placed over the polypyrrole layer. Each step is very important in order to obtain a micro ion-selective electrode with good analytically important parameters.

**Preparation of disk-in-glass microelectrodes**

Three different electrode materials were used to prepare disk-in-glass microelectrodes: carbon fiber, gold and platinum. The Au and Pt wires and carbon fibers were sealed in soft glass tubes, about 8 cm long with id about 1 mm and od about 2 mm. Prior to use the glass tubes were carefully cleaned by soaking in a 1 + 1 v/v mixture of sulfuric acid and 30% H₂O₂ for at least 2 h and then washed thoroughly with de-ionized water and dried at 120 °C in a thermostated box. Before placing the wires inside the lumen of the capillary, they were soaked in 2 M HNO₃ and rinsed with water and acetone.

The glass was sealed around the wire or fiber 3–4 mm from the tip. The microelectrodes were inspected under an optical microscope to determine the quality of sealing (bubbles, good adherence of the glass to the wire, etc.). The sealed end was polished with sandpaper until the end of the wire was exposed (gradually with finer and finer sandpaper on coming closer to the end), and then successively with 1, 0.3 and 0.05 μm aluminum oxide suspensions. After the last polishing step the electrodes were immersed in an ultrasonic bath for 30 s. Polishing with the aluminum oxide suspension was repeated each time before re-use of the disk electrodes. The electrical connection between the copper wire and the disk was made with mercury or silver epoxy glue. Finally, the sealed end of the glass tube was conically bevelled around the measuring disk. This is especially important when the microelectrode is used in SECM measurements.

The performance of the microelectrodes was checked by cyclic voltammetry at different scan rates in the positive polarization range using 4 mM potassium hexacyanoferrate(II) solution in 1 M KCl and in the negative potential range using 2 mM ruthenium hexamine trichloride solution in 0.1 M KCl. Cyclic voltammograms of two microelectrodes [diameter 8 μm (C) and 5 μm (Pt)] in 4 mM K₃[Fe(CN)₆] in 1 M KCl solution are shown in Fig. 1. Only those microelectrodes which gave a theoretical step-shaped voltammogram were selected for further work. The shape of the voltammograms obtained with the 300 μm gold electrode resembled more the shape of the voltammograms obtained with macroelectrodes.

**Polypyrrole film preparation**

PPy films were prepared by electrochemical polymerization on the substrate electrode from a solution of 0.5 M pyrrole in 1.0 M KCl by applying a constant potential of +800 mV versus Ag/AgCl for 30 s. The solution was purged for at least 15 min with nitrogen before polymerization and nitrogen was passed over the solution during polymerization.

After polymerization, the microelectrodes were rinsed with de-ionized water and cyclic voltammograms of the polypyrrole film were recorded in 0.1 M KCl. The solution was purged with nitrogen prior to the measurement. In accordance with the literature, we found it important, from the point of view of the conditioning time in an anion containing solution, to stop the cycling at a potential more positive than the oxidation potential of the film in order to have the PPy film in a doped state. The electrodes were then conditioned by soaking them in 0.1 M KCl for 24 h and then analyzed by cyclic voltammetry using a scan rate of 50 mV s⁻¹.
for at least 10 h before the potentiometric response for chloride was checked.

**Ion-selective membranes**

Ion-selective membranes of two different compositions were prepared. Membrane cocktail A was composed of 4% m/m BME-44, 10% m/m PVC, 86% m/m oNPOE and 70 mol% KTpClPB and membrane cocktail B contained 2% m/m BME-44, 32% m/m PVC, 66% m/m oNPOE and 70 mol% KTpClPB. The membrane components were dissolved in an appropriate amount of THF. About 1 μl of ion-selective cocktail was applied precisely on the top of the PPY film under a stereo microscope with a microsyringe. The electrodes were then allowed to dry, usually overnight, in an upright position. When not in use the ion-selective membrane coated PPY solid contact electrodes were stored in 0.1 M KCl. The schematic design of the microelectrode is shown in Fig. 2.

**Preparation of coated wire electrodes**

In order to compare the characteristics of the ion-selective microelectrodes with and without the PPY charge mediating layer, coated wire electrodes were also prepared. In this case, the potassium-selective membrane cocktail A was applied directly on the carbon fiber microelectrode (diameter 30 μm) in the same way as described for the PPY solid contact K⁺ selective electrodes.

Subsequently in this paper, the following convention will be used to name the electrodes: size substrate/solid contact/ionophore, e.g., 5 μm Pt/PPy/BME-44.

**Results and discussion**

**Voltammetric and potentiometric response of the polypyrrole films**

To confirm the presence of the Cl⁻ doped PPY film on the microelectrode surface, cyclic voltammograms in 0.1 M KCl were recorded. The shape of the cyclic voltammograms agreed well with that of small, mobile anion doped PPY films deposited on macroelectrodes.³²

Before covering the polypyrrole film with the ion-selective membrane, the potentiometric chloride response of the different polypyrrole coated microelectrodes was measured in 10⁻²–10⁻¹ M KCl solutions. The ionic strength of the solutions was not adjusted to a constant level because of the low selectivity of the polypyrrole electrodes.³¹ No corrections for activities or liquid junction potentials were made, because our interest was to see only qualitatively the anionic sensitivity of the microelectrodes.

For all types of electrodes the slope values of the electrode calibration graphs were above 50 mV per decade in the concentration range 10⁻⁴–10⁻¹ M KCl. This value correlates well with the sensitivity of conventional macroelectrodes prepared in the same way as the microelectrode (Fig. 3).³³ The fact that the anion sensitivity is less than the theoretical —9.16 mV per decade (at 25 °C) suggests that the response of the polypyrrole electrodes may not be purely anionic.³⁰

**Potentiometric characterization of the solid contact potassium-selective electrodes**

The ionic response of the potassium-selective membrane coated electrodes, with a PPY solid contact, was measured in a series of KCl solutions. It was found that the potassium sensitivity of the microelectrodes varied from electrode to electrode, but they were always higher than 50 mV per decade in the concentration range 10⁻⁵–10⁻¹ M KCl. As an example, the calibration graph for a 5 μm Pt/PPy/BME-44 microelectrode in comparison with that of a 3 mm Pt/PPy/BME-44 macroelectrode is shown in Fig. 4. The mean activity coefficients of the electrolytes were calculated with the Debye–Hückel equation and the liquid junction potentials were estimated with the help of the Henderson formalism.

Fig. 2 Schematic design of the polypyrrole solid contact potassium-selective microelectrode.

![Fig. 2](image)

Fig. 3 Potentiometric response of different 5 μm (1, 2, 3) and 3 mm (4, 5) Pt/PPy electrodes in KCl solutions.

![Fig. 3](image)

Fig. 4 Potentiometric response of a 5 μm Pt/PPy/BME-44 microelectrode (1) and a 3 mm Pt/PPy/BME-44 macroelectrode (2). The calibration graphs were obtained by averaging the daily calibration graphs during 1 month. The average slopes relevant to the concentration range 10⁻¹–10⁻⁴ M were 52.3 mV per decade (r = 0.999) for the 5 μm Pt/PPy/BME-44 microelectrode and 54.1 mV per decade (r = 0.999) for the 3 mm Pt/PPy/ BME-44 macroelectrode.

![Fig. 4](image)
The potential response function of the microelectrodes remained stable for longer than 2 weeks. The within-day average slope values of the calibration graphs for a 5 μm Pt/PPy/BME-44 microelectrode and that for a 3 mm Pt/PPy/BME-44 macroelectrode were proved to be not significantly different after performing a t-test at the 0.05 significance level. This proves that the sensitivities of the micro- and macroelectrodes are similar and no sensitivity decrease is observed owing to the decrease in size of the electrodes.

For comparison, the calibration properties of the coated wire electrode were also determined in KCl solutions and a cationic response was obtained at concentrations higher than $10^{-3}$ M. The potential values were found to drift and they did not stabilize within a few minutes as did the electrodes with the PPy solid contact. This clearly shows the usefulness of the PPy film as a solid contact between the metal substrate and the PVC membrane in the case of the microelectrodes. This is in good agreement with the observations reported for macroelectrodes with a PPy solid contact and a sodium-selective PVC membrane.

The selectivity coefficients of the potassium microelectrodes were determined for Na$^+$, Li$^+$, NH$_4^+$ and Cs$^+$ using the separate solution method at the 0.1 m concentration level. The relevant selectivity data determined are given in Table 1 and clearly show that microelectrodes coated with ion-selective membrane A have almost no selectivity for potassium in the presence of most of the interfering ions tested. In contrast, electrodes sensitized with membrane composition B showed improved selectivity. The selectivity data thus determined are comparable to the selectivity coefficients reported in the literature for conventional macroelectrodes using the same ion-selective membrane composition, and allow the electrodes to be used for potassium determination in biological samples. This observation clearly indicates that the polypyrrole solid contact does not have any negative effect on the selectivity of the microelectrodes. The poor selectivity coefficients obtained for electrodes prepared with membrane composition A are probably due to the ion-selective layer smearing off because of the high plasticizer content. This effect was found to be more significant for electrodes with thin membrane layers. Membrane composition B was used in subsequent studies.

On renewal of the ion-selective membrane, the microelectrodes had to be polished as described under Experimental. A new PPy film was then polymerized and the ion-selective cocktail was applied. Just dissolving the PVC membrane in THF and applying a new membrane gave poor results.

The stability and reproducibility of the potentiometric response were evaluated on the basis of the slopes of the calibration graphs for the microelectrodes. The stability of the electrodes during the first 10 d was checked daily, and thereafter randomly. The slopes remained fairly constant (RSD = 5.9%).

### Table 1

<table>
<thead>
<tr>
<th>Electrode</th>
<th>$j = \text{Li}^+$</th>
<th>$j = \text{Na}^+$</th>
<th>$j = \text{Cs}^+$</th>
<th>$j = \text{NH}_4^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane composition A.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt 5 mm</td>
<td>-3.88</td>
<td>-3.02</td>
<td>-3.14</td>
<td>-3.02</td>
</tr>
<tr>
<td>C 30 mm</td>
<td>-1.03</td>
<td>-0.48</td>
<td>-1.64</td>
<td>-0.71</td>
</tr>
<tr>
<td>C 8 mm</td>
<td>-1.64</td>
<td>-0.71</td>
<td>-1.86</td>
<td>-0.85</td>
</tr>
<tr>
<td>Membrane composition B.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt 5 mm</td>
<td>-3.32</td>
<td>-2.94</td>
<td>-2.40</td>
<td>-2.40</td>
</tr>
<tr>
<td>C 30 mm</td>
<td>-2.88</td>
<td>-2.73</td>
<td>-2.24</td>
<td>-2.04</td>
</tr>
<tr>
<td>Au 30 mm</td>
<td>-3.44</td>
<td>-3.13</td>
<td>-2.23</td>
<td>-2.02</td>
</tr>
</tbody>
</table>

The data were determined with the separate solution method at 0.1 M concentration level.

over a time period longer than 2 weeks. This is valid for all of the microelectrodes which were prepared, independently of their size or material.

### Study of the use of the microelectrodes in flowing systems

In order to study the dynamic characteristics and generally the behaviour of the microelectrodes under flowing conditions, a wall-jet cell based flow injection system was set up, as presented in Fig. 5. The all-solid-state microelectrode design (cylindrical shape) allowed the microelectrodes to be fixed easily to the wall-jet potentiometric cell.

The flow injection technique gives the possibility of determining the response time of the microelectrode incorporated potentiometric cell if a large volume of solution of appropriate composition is injected into the system. In our experiment, $10^{-3}$ M KCl solution was used as the carrier solution and 1 ml of 0.1 M KCl solution was injected. The injection of a large volume of solution was necessary to achieve a steady-state potential signal after injection. We took as the response time the time required for the potentiometric cell to achieve 95% of the steady-state potential value, corresponding to the concentration step. Fig. 6 shows the potential versus time recording due to the concentration step employed, from which a response time of 3.7 s was calculated. As Fig. 6 shows, there is an apparent disagreement between the longer recovery period (falling part of the curve) and the sharp rising part of the curve from which the response time was estimated. The longer recovery time is due to the wash-out time of the measuring cell. As longer sample plugs of high concentration were introduced and bearing in mind the logarithmic dependence of the signal on the concentration, the wash-out time of the cell can be easily 30 s or more. Hence the rising part of the curve is more appropriate for the determination of the response time.
To study the potentiometric sensitivity of the microelectrodes under flow injection conditions, 0.1 M CaCl₂ solution was used as the carrier solution (since the potassium electrode hardly responds to Ca²⁺), at a flow rate of 2 ml min⁻¹, into which 250 μl portions of KCl solutions of different concentrations (10⁻¹⁻¹0⁻⁶ M) were injected consecutively. The calibration graph obtained by plotting the peak height versus the logarithm of the potassium activity is shown in Fig. 7. The slope of the calibration graph is close to that obtained in batch measurements (also included in Fig. 7), which is an indication that these electrodes have good dynamic characteristics (response time) and can be used successfully as a sensor in transient techniques such as a flow injection analysis system. These results also show that the microelectrodes have the good mechanical resistance needed for their use under flow conditions.

Scanning electrochemical microscopic studies with the new potentiometric microelectrodes

A challenging application for the microelectrodes is to employ them as a measuring tip in SECM measurements. Only stable and very small electrodes with low noise, short response time and especially the mechanical resistance and robustness of the tip gave the three-dimensional image of the K⁺ concentration profile over the target. The image thus obtained (Fig. 8) demonstrates the capability of this novel design of potentiometric microelectrode as an SECM tip.

In the SECM measurements, a glass capillary filled with KCl-containing agarose gel was employed as an ion-concentration profile generating target. The size of the capillary and the composition of the agarose gel were optimized in order to have an appropriate flux for the potassium ion diffusion from the capillary into the electrochemical cell. Hence the SECM measurements were carried out under steady-state conditions. For our studies a 70 μm id glass capillary filled with 0.1 M KCl in 2% agarose was prepared and used. The capillary was introduced from the bottom of the electrochemical cell, filled with de-ionized water, through a small hole and it was fixed in an upright position.

During one scan the ion-selective microelectrode was moved in the XY plane over the target and simultaneously the measured potential values were recorded and rendered to the geometrical coordinates of the tip. The movement of the tip was controlled by personal computer. The graphical representation of the potential values recorded versus the geometrical coordinates of the tip gave the three-dimensional image of the K⁺ concentration profile over the target.

The image thus obtained (Fig. 8) demonstrates the capability of this kind of microelectrode as a measuring tip in SECM measurements.

References


