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Conducting Polymer Films as Model Biological Membranes. Electrochemical and Ion-Exchange Properties of PPy and PEDOT Films Doped with Heparin*

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The interaction of calcium, magnesium potassium and sodium cations with heparin (Hep), a highly negatively charged glycosaminoglycan, was studied by using conducting polymer (CP) films. The CP-Hep films were obtained by electrochemical deposition of poly(pyrrole) (PPy) or poly(3,4-ethylenedioxythiophene) (PEDOT). In order to induce magnesium or calcium sensitivity, all films were conditioned in alkaline solution containing magnesium or calcium ions, respectively. After conditioning, the potentiometric response towards calcium or magnesium ions with a close-to-Nernstian slope was observed. The calcium or magnesium sensitive CP-heparin films do not respond to Na or K ions, but the response towards Mg or Ca ions was almost identical. A distinct difference in dynamic open-circuit response to calcium or magnesium ions was observed and its relevance to biological membrane processes is stressed. CP-heparin films can be used for testing an ion exchange as well as transport processes that occur in biological membranes.

Key words: poly(pyrrole) (PPy), poly(3,4-ethylenedioxythiophene) (PEDOT), heparin, membrane potential, action potential, biological cell membrane

It is well known that conducting polymers (CPs) in the oxidation process during electrodeposition are easily doped with small inorganic anions and consequently exhibit anionic open-circuit sensitivity. Cationic sensitivity can be observed when the CP films doped with bulky immobile anions are reduced in the presence of mobile cations [1]. It was shown that the cationic sensitivity may be enhanced and stabilized by using metal-complexing ligands from the group of metallochromic indicators as dopants [2–4]. This happens because the bulky dopant anions retain in the polymer film their complexing properties known from water chemistry providing the unique possibility of forming CP films doped with big and biologically active anions such as heparin (Hep) [6,7] or adenosine triphosphate (ATP), as shown recently [5,8,9]. Such films may be used as model biological membranes to inspect processes important for membrane potential formation or membrane transport.

In this work, a method to obtain CPs films doped with heparin is described.

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* Dedicated to Prof. Dr. Z. Galus on the occasion of his 70th birthday.
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Poly(pyrrole) (PPy), one of the most frequently used CPs, and poly(3,4-ethylenedioxythiophene) (PEDOT) are applied as the polymer matrix – the latter recognized to be the most chemically stable organic conducting polymer currently available [12,13].

Heparin is a highly sulfonated glycosaminoglycan constituted by disaccharic repeating units of D-glucosamine (GlcN) and L-iduronic acid (IdUA) (Fig. 1). It contains many covalently linked sulfate and carboxylic acidic groups. In sodium salt of heparin, the acidic protons of the sulfate units are partially replaced by sodium ions.

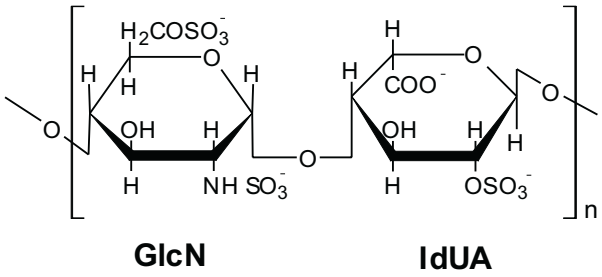

Figure 1. Sequence scheme of a heparin chain; GlcN – D-glucosamine, IdUA – L-iduronic acid.

Heparin is widely used in medicine as an anticoagulant and antithrombotic agent. Heparin also activates lipoprotein lipase and inhibits the growth and replication of the human immunodeficiency virus (HIV). The activity of heparin is modulated by calcium ions. In particular, calcium moderates the anticoagulant activity of heparin by affecting heparin-stabilized tryptase in human mast cells, and by influencing the role of heparin in thrombin inhibition. The interaction between heparin and calcium cations is strongly electrostatic; heparin has the highest negative charge density of any known biological macromolecule, carrying an average of ~ 80 negative charges at physiological pH [10]. Owing to its high negative charge density, heparin is a polyelectrolyte, *i.e.* a fraction of its negative charge is neutralized by bound counterions.

Purified heparin is routinely added to blood samples obtained for clinical analysis and to blood donated for transfusion to prevent clotting. As heparin binds very strongly to calcium ions, the long usage of heparin as an anticoagulant can eventually lead to osteoporosis [11]. Therefore, it is extremely important to understand the interactions between calcium ions and heparin. For this reason, an attempt to deposit conducting polymer films doped with heparin to allow for the exposure of calcium and magnesium ion interaction with heparin was undertaken. The focus was on the tendency with time for membrane potential to form on the films in the latter processes. In general, it is thought that conducting polymer films doped with biologically active ligands can serve in providing a good model membrane to study the interactions and processes occurring in real biological membranes.

EXPERIMENTAL

Chemicals. Heparin sodium salt from bovine intestinal mucosa (Fluka) or Clexane (enoxaparinum natricum, 100 mg/1 ml from Rhone–Poulenc Rorer, France) were used as received. Pyrrole (Merck) was purified by double distillation under argon gas and then stored under argon at low temperature and protected from light. The monomer 3,4-ethylenedioxythiophene (EDOT, >97%) was obtained from Bayer AG. The TES buffer, N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (Fluka), $CaCl_2$ p.a (POCh), $MgCl_2$ p.a (POCh), CaO p.a (POCh), MgO p.a (POCh), NaCl p.a (Merck), KCl p.a (Sigma Chemicals) were used as received. Water, redistilled from quartz was used to prepare the solutions. All the solutions with a concentration lower then 0.01 M were prepared just before use.

Instrumentation. Electrochemical polymerization of poly(pyrrole) (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT) was carried out using an EA-9C type electrochemical analyzer (MTM Poland). The electropolymerization was performed in a single-compartment, three-electrode electrochemical cell. The working electrodes, to be covered with conducting polymer film, were a platinum (Pt) or glassy carbon (GC) disc electrode (area 0.03 cm^2 or 0.07 cm^2 respectively), as well as a conducting glass (ITO) sheet with an area of about 1 cm^2. The reference electrode was an Ag/AgCl/sat.KCl electrode, which was connected to the cell *via* a bridge filled with supporting electrolyte solution. A Pt wire or Pt sheet (area about 2 or 5 cm^2 respectively) was used as an auxiliary electrode. All the potentiometric measurements were performed using a homemade 8-channel set-up. The input impedance was greater than 10^{13} ohms and the input current was lower then 0.1 pA for each of the 8 inputs as well as for the reference electrode input. The multi-channel potential-meter was coupled to a personal computer equipped with a 16-bit resolution data acquisition card CIO-DAS802/16 (ComputerBoards) and custom-made software. An Ag/AgCl/3M KCl electrode was used as the reference electrode. All the experiments were performed at 22°C. The Energy Dispersive Analysis of X-ray (EDAX) measurements were carried out using a Jeol 5400 scanning microscope (Jeol, Japan). The Electron Spectroscopy for Chemical Analysis (ESCA) measurements were preformed with Physical Electronics Quantum 2000 ESCA-spectrometer equipped with monochromatized Al-X-ray source. The size of the analyzed area was 100 μm in diameter and the analyzing depth was about 2–5 nm depending on the investigated element. The CP-heparin films were deposited on the ITO sheets, Pt sheets as well as on the graphite disc for such measurements.

Preparation of CP-heparin electrode. Before polymerization of the pyrrole or 3,4-ethylenedioxythiophene (EDOT), the Pt or GC discs were finally polished with 0.3 μm alumina and carefully rinsed with water. Next, the electrode to be covered with CP was immersed in ethanol and placed in an ultrasonic bath for about 10 minutes. Just after this, the electrode was rinsed with water and immediately immersed in the solution used for the electropolymerization. The ITO sheets were manually cleaned, then immersed in ethanol and placed in an ultrasonic bath for about 10 minutes before use. Electrodeposition was performed from the solution containing 16 mg/ml of heparin and 0.1 M pyrrole or 40 mg/ml of heparin and 0.01 M EDOT. The solution was saturated with argon for at least 20 minutes before polymerization, and argon gas was passed over the solution during electropolymerization. The PPy and PEDOT films were deposited potentiostatically on the working electrode. Prior to electropolymerization, the cyclic voltammetry measurements were performed and the potential value required for monomer oxidation was determined. In the case of PPy, the potential used was constant and equal to 0.750–0.850 V (*vs.* Ag/AgCl) or was pulsed between 0–0.750 V with equal pulse width. The deposition time was 600–1800 sec. In the case of EDOT deposition, the constant potential equal to 0.960 V was used and the deposition time was equal to 300 sec. Prior to the potentiometric measurements, the films were usually conditioned in a mixture of 0.1 M calcium chloride and calcium hydroxide (pH of about 10.5–11.5) or in saturated $Mg(OH)_2$ solution with a pH of about 10.5. Afterwards, the potentiometric response in $CaCl_2$, $MgCl_2$, KCl, or NaCl solutions was checked. As a rule, a cationic response with a close-to-Nernstian slope for a tenfold change in the activity of calcium or magnesium ion was observed for the PEDOT films already after 1 day of soaking. For the PPy films, the required soaking time was longer, and equal to 1 week.

RESULTS AND DISCUSSION

The presence of the sulfur peak in the EDAX analysis of PPy-heparin films (Fig. 2a) proves that heparin dopes the films formed during electrodeposition. The EDAX analysis taken after soaking of the PPy-Hep films with alkaline calcium solution proves that the heparin in the film is able to complex calcium (Fig. 2b) ions.

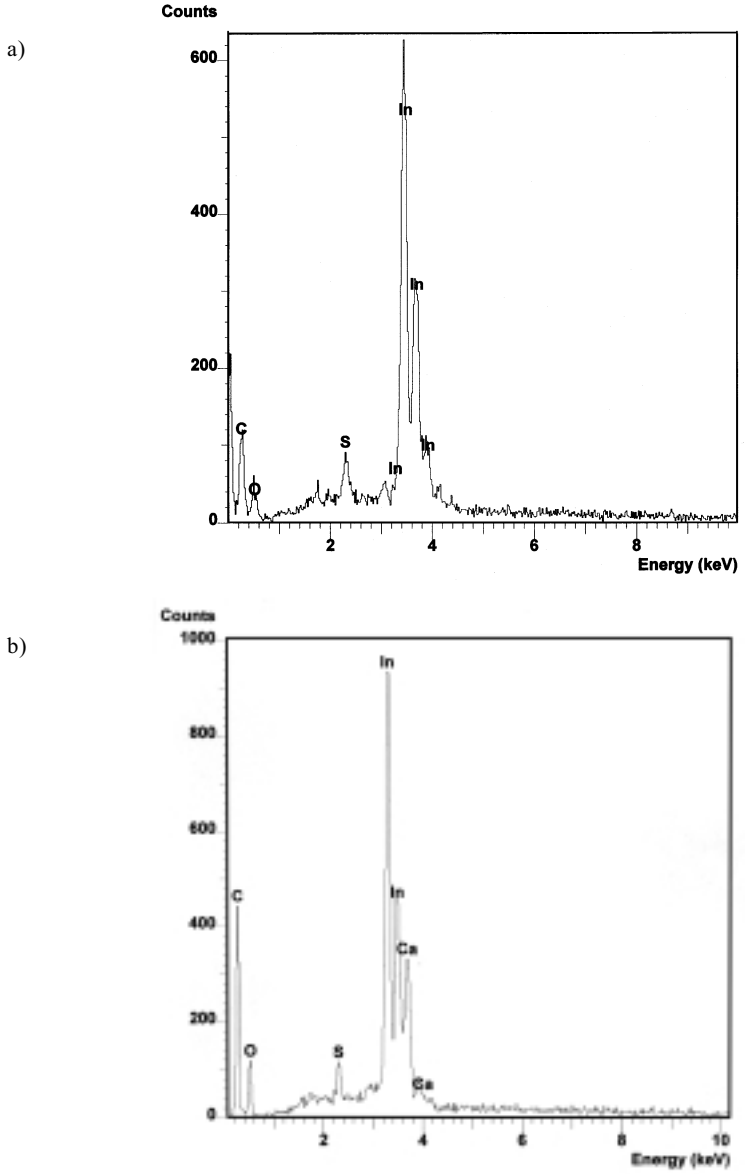

Figure 2. The EDAX spectra for PPy-heparin film before soaking (a), and after soaking with alkaline calcium solution (b).

Similar results were obtained for magnesium ions. It should be noted that prior to EDAX or ESCA measurements the sensitivity towards calcium or magnesium ions was always checked and proved.

In the case of PEDOT-Hep films, the presence of heparin was proved by the nitrogen peak, visible in the ESCA spectra (see Fig. 3a). After soaking the PEDOT-heparin films with alkaline calcium or magnesium solutions, additional peaks of calcium (Fig. 3b) or magnesium (Fig. 3c) appeared, thus proving that calcium or magnesium was present in the CP-Hep films, which indicates the formation of calcium and/or magnesium complexes with heparin inside the PEDOT-Hep films. The freshly deposited and unsoaked PPy-heparin or PEDOT-heparin films were practically ion-insensitive and the slopes of the calibration curves towards calcium, magnesium, sodium and potassium ions were close to zero.

Almost theoretical potentiometric sensitivity towards magnesium or calcium ions was observed after adequately long film soaking. The potentiometric sensitivity for magnesium and calcium associated with the presence of those ions in the conducting polymer film has been previously reported for PPy doped with metallochromic compounds and was attributed to the metal-binding properties of the doping ions [2–4]. The slope value was practically unchanged even if soaking time was extended up to 1 year. As an example, Fig. 4 shows the Ca sensitivity of unsoaked PPy-heparin film (curve "without soaking") as well as Ca sensitivity after 3 months, 7 months and 12 months of soaking with alkaline calcium solution (curves 3, 7 and 12, respectively). Similar results were obtained for PPy-heparin films sensitized towards magnesium as well as PEDOT-Hep films sensitized towards calcium or magnesium. As an example, Fig. 5 shows such calibration curves for PEDOT-Hep film and magnesium ions.

The CP-Hep films sensitized towards calcium or magnesium became practically insensitive towards sodium or potassium ions. On the other hand, the sensitivity of both groups of films towards calcium or magnesium ions was similar. According to our previous observation [4–5], this proves that the stability constant of calcium or magnesium complexes with heparin are similar and much bigger than those for sodium or potassium complexes. As an example, Fig. 6 shows the potential change for calcium-sensitive PPy-Hep film caused by a sodium, potassium, or magnesium concentration increase in 0.0001 M $CaCl_2$ solution.

In spite of similar sensitivity, the transitory potential provoked by the changes in bulk concentrations of magnesium or calcium ions was quite different and independent of the initial film sensitivity (*i.e*. it was similar for calcium and magnesium-sensitive films). The overshot type response was recorded if the calcium concentration was changed, whereas the monotonic response was a result of magnesium concentration changes.

As an example, Fig. 7 shows the transitory potential for calcium-sensitive PEDOT-Hep film caused by calcium or magnesium concentration changes. It should be noted that, if the calcium response was to be checked, the investigated solution contained also magnesium salt with a constant and equal to 0.0001 M concentration.

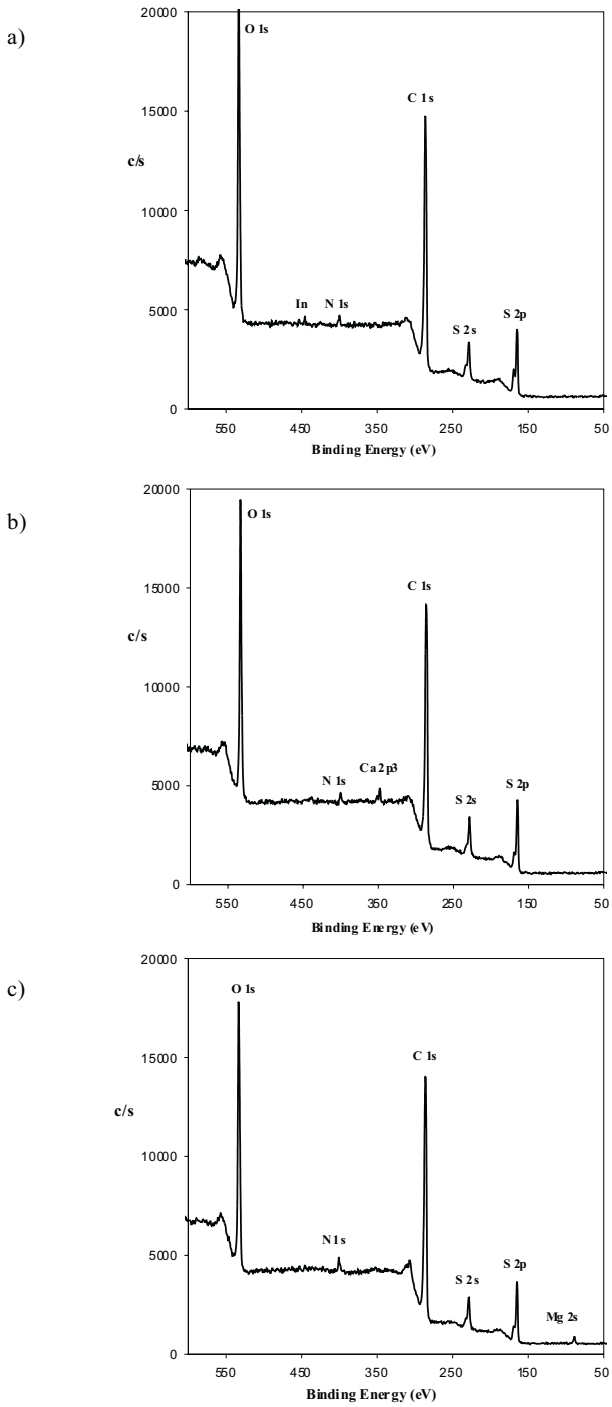

Figure 3. ESCA spectra for PEDOT-heparin films before soaking (a), after soaking with alkaline calcium solution (b), and after soaking with saturated magnesium hydroxide solution (c).

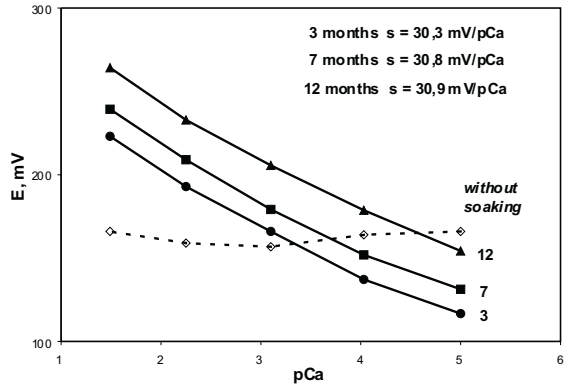

Figure 4. The calibration curves for freshly deposited PPy-heparin film (curve "without soaking") and after indicated soaking period with alkaline calcium solution. The determined slope values are inserted.

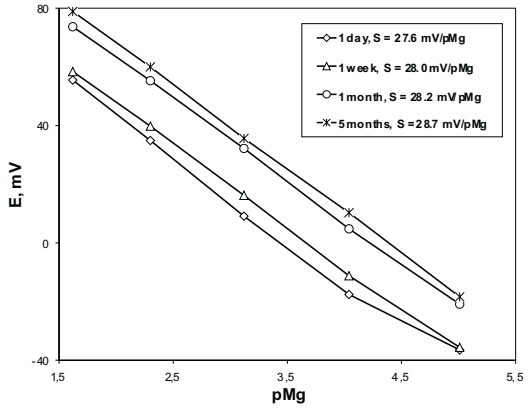

Figure 5. The calibration curves and determined slope value for PEDOT-heparin film taken after indicated soaking period with saturated magnesium hydroxide solution.

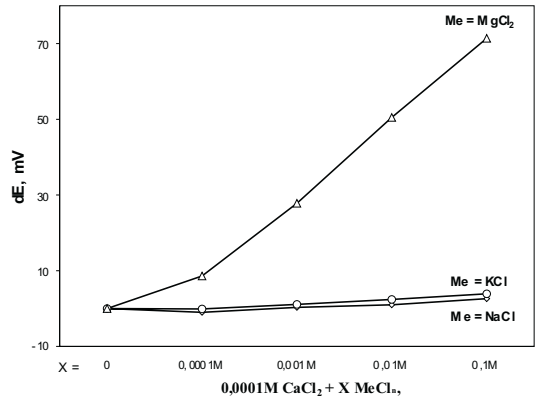

Figure 6. The selectivity test towards sodium, potassium and magnesium ions of the calcium-sensitive PEDOT-heparin film.

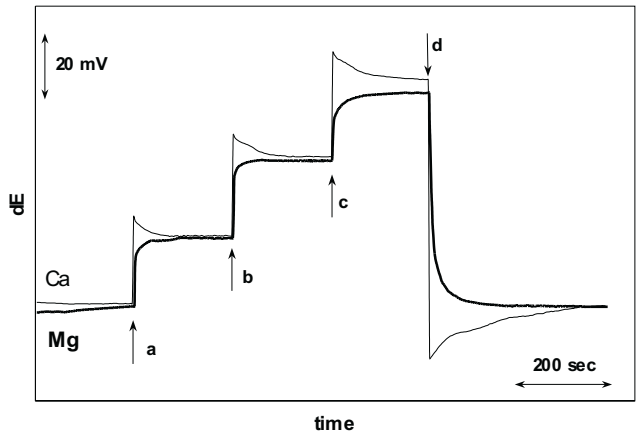

Figure 7. Potential response of calcium-sensitive PEDOT-heparin film caused by magnesium (the bold curve) or calcium (the thin curve) concentration changes. The initial solution contains both $CaCl_2$ and $MgCl_2$ each with concentration of 0.0001 M. Concentration of $MgCl_2$ or $CaCl_2$ was changed to: 0.001 M (a), 0.01 M (b), 0.1 M (c) and again to 0.0001 M (d).

On the other hand, if the magnesium response was checked, then the investigated solution also contained calcium salt with a constant and equal to 0.0001 M concentration. The results obtained with buffered solutions were almost identical, which excludes any significant influence of hydrogen ions. Figure 8 shows the transitory potential for magnesium-sensitive PEDOT-heparin film caused by calcium or magnesium concentration changes. In order to exclude the pH influence in all solutions during measurements, the pH was buffered by TES to 7.4.

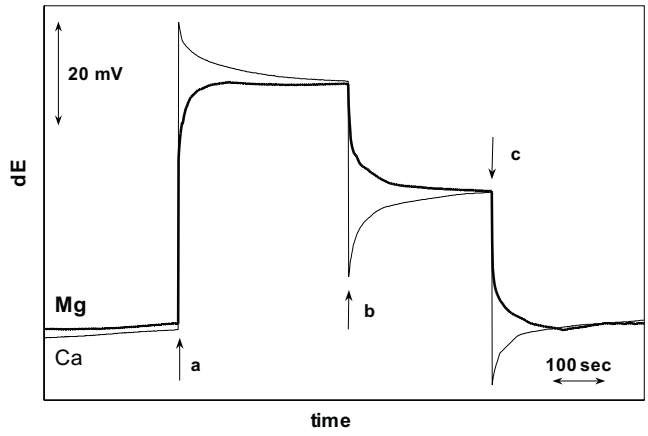

Figure 8. Potential response of magnesium-sensitive PEDOT-heparin film caused by magnesium (the bold curve) or calcium (the thin curve) concentration changes. All measurements were performed for solutions buffered with TES, pH = 7.4. The initial solution always contains both $CaCl_2$ and $MgCl_2$ with concentrations of 0.0001 M each. Concentration of $MgCl_2$ or $CaCl_2$ was changed to: 0.01 M (a), 0.001 M (b), and again to 0.0001 M (c).

The overshoot type of potential-time response resulting from the changes in calcium concentration indicates a fast calcium exchange process in the membrane of the calcium electrode, in contrast to monotonic potential changes observed when the magnesium concentration was changed while the calcium remained constant. This membrane potential-time behaviour was previously observed for magnesium ion-selective PVC-based electrode membranes with ETH 5220 as a neutral carrier. This behaviour was ascribed to kinetic discrimination during competitive Mg^{2+} and Ca^{2+} ion-exchange on the membrane surface [14,15]. Similar ion discrimination observed by the authors for heparin as the active site in Mg^{2+} and Ca^{2+} ion-exchange indicates that, in real biological processes, the availability of calcium and magnesium ions on heparin membrane sites may be controlled in a similar way, depending on membrane potential and time. It is noteworthy that the same discriminating mechanism for Mg^{2+} and Ca^{2+} ions was recently found by the authors for poly(pyrrole) films doped with adenosine-tri-phosphate (ATP), which plays here the role of the active ion-exchange site [5]. In both these cases, the potential-time behaviour can be interpreted by the diffusion-layer model (DLM) [16,17]. According to this model total equilibrium may be disturbed by changing the bulk concentration of species engaged in an ion-exchange:

$$\overline{\text{CP-Hep}(Mg^{2+})} + Ca^{2+}_{sol} \xleftrightarrow{K_{Mg^{2+},Ca^{2+}}} \overline{\text{CP-Hep}(Ca^{2+})} + Mg^{2+}_{sol}$$

or by applying external electric potential, *e.g.* in a form of pulse.

A change of total equilibrium provokes equilibration process in which the local concentration of ions participating in ion-exchange process at the membrane | solution interface differ from these in the bulk until a new total equilibrium is reached. Due to the local concentration changes electric potential drop at the interface will correspondingly change, either monotonically or *via* overshoots and are characterized numerically by DLM [16, 17].

CONCLUSIONS

Poly(pyrrole) and PEDOT films doped with heparin anions were obtained by electrodeposition from watery solution. After soaking with alkaline calcium or magnesium solution, adequate complexes were formed inside the films, and the presence of heparin as well as calcium or magnesium ions was confirmed by EDAX or ESCA measurements. The potentiometric sensitivity towards calcium and magnesium ions became close to Nernstian after soaking, and the response was not affected by sodium or potassium ions. Induced calcium or magnesium sensitivity remained unchanged even if as long as a 1-year soaking period was used. Transitory behaviour of the films is observed during equilibration provoked by a change in bulk magnesium or calcium concentration. Such behaviour is ascribed to a different rate of magnesium and calcium ion transfer between the bulk of the solution and membrane, which is thought to be of primary importance in membrane processes in biological cells.

Acknowledgments

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