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Conducting polymers in modelling transient potential of biological membranes

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Abstract

The possibility of using conducting polymer (CP) films doped with biological ligands as artificial biological membranes to study potential formation mechanisms is presented. Calcium and magnesium ion-binding anionic sites — asparagine, glutamine, adenosinotriphosphate and heparin are incorporated into the poly(pyrrole) film during electrochemical polymerization. This approach allows the competitive calcium–magnesium ion-exchange to be inspected by open circuit measurements. After a close-to-Nernstian sensitivity of the CP membranes was induced by soaking in alkaline solutions of calcium or magnesium, dynamic experiments were performed by a change in the bulk concentration of magnesium or calcium ions. A characteristic transitory potential response, though distinctively different for the calcium and magnesium ions, was observed and explained using the diffusion layer model (DML).

Keywords: Poly(pyrrole); Ion-exchange kinetics; Ca–Mg competition; Potential transients

1. Introduction

The change in membrane potential with time is of fundamental importance in cell biology. It was recently shown in our studies that conducting polymer (CP) films doped with biological ligands (BLs) may be used as model biological membranes to study the mechanism of membrane potential formation [1–3]. In particular, the CP–BL–Me films may be used to study the competitive binding of cations to biologically active ligands and to observe the resulting effect on transient membrane potential during equilibration.

The ligands in focus of our research, adenosinotriphosphate (ATP), heparin (Hep) and two amino acids — asparagine (Asn) and glutamine (Gln), competitively bind calcium and magnesium ions and thus play an important role in calcium and/or magnesium-dependent biological membrane processes [4]. In particular, ATP takes part in active membrane potential formation, Hep in the anticoagulation process, and Asn and Gln in the voltage–ligand gated influx on calcium ions via the NMDA channels [5–7].

The following methodology is accepted for applying CPs as model biological membranes. In order to obtain the membranes (CP–BL–Me), first ATP, Hep, Asn or Gln are introduced into the CP (here: poly(pyrrole) (PPy)) matrix during electropolymerization. Next, the calcium or magnesium potentiometric sensitivity is induced by soaking in an alkaline solution of calcium or magnesium ions until close-to-Nernstian sensitivity for the films under equilibrium was attained. The films are then used to monitor the equilibration processes induced by the change in bulk concentration of magnesium or calcium ions. The resulting transitory potential response is recorded and characteristic potential transients observed are theoretically interpreted.

In this report, we summarize our observations on the interaction of calcium and/or magnesium ions with biologically active ligands (sites), and provide a generalized picture and

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quantitative model of the influence of the ion-binding and ion-exchange on membrane potential formation.

2. Experimental

2.1. Reagents

Pyrrole (Merck) was purified by double distillation under argon and then stored under argon at low temperature and protected from light. The L-Asparagine, L-Glutamine, adenosine 5-triphosphate sodium salts, heparin sodium salt from bovine intestinal mucosa, sulfosalicylic acid and the TES buffer, (*N*-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid) were obtained from Fluka. The other reagents were purchased from J.T Baker. All chemicals used were of analytical grade. Doubly distilled and freshly deionized ELGA water (resistivity 18.2 MΩ cm) was used throughout the work.

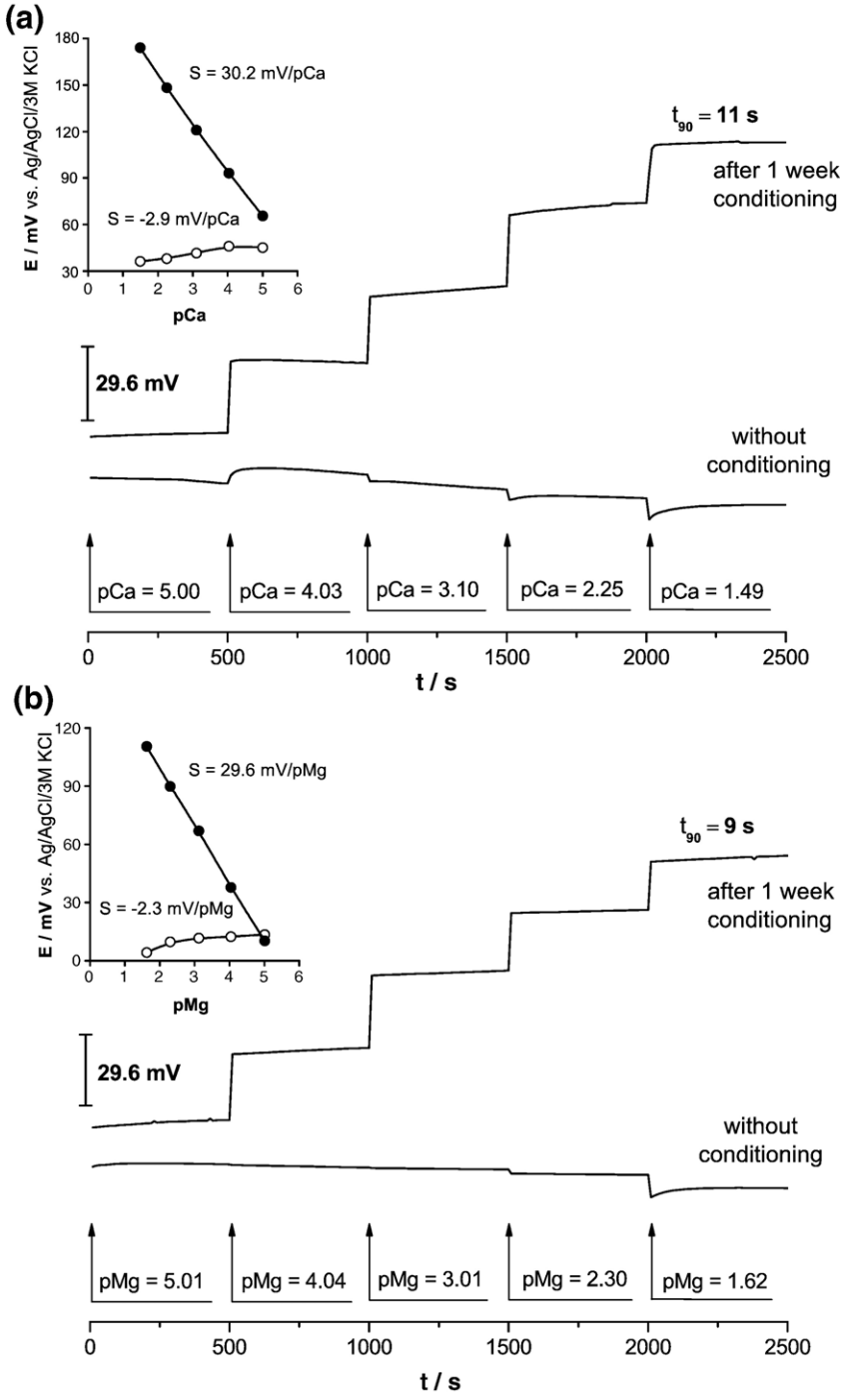

Fig. 1. The comparison of the potentiometric responses recorded in $CaCl_2$ (a) and $MgCl_2$ (b) solutions for PPy–Asn films before (open circles) and after (full circles) conditioning in alkaline Ca (a) and Mg (b) solutions (where: $pX = -\log a(X)$ for X = Ca, Mg).

2.2. Apparatus

Potentiostatic and potentiodynamic synthesis of polymer films on GC and ITO electrodes was carried out using an Autolab general Purpose System (AUT20.Fra2-Autolab, Eco Chemie, B.V., The Netherlands) connected to a conventional, three-electrode cell. The working electrode was a glassy carbon disk with an area 0.07 cm^2 or conducting glass pieces with an area of about 1 cm^2 (ITO, Lohja Electronics, Finland, used for the XPS experiments). The reference electrode was an Ag/AgCl/sat.KCl electrode connected to the cell via a bridge filled with supporting electrolyte solution, and a glassy carbon (GC) rod was used as the auxiliary electrode. The solutions used for polymerization contained 0.1 M pyrrole and an electrolyte that provided the doping ion. Electropolymerization was performed in solutions saturated with argon at room temperature.

The potentiometric measurements were made with a homemade multi-channel set-up. The reference electrode was an Ag/AgCl/3M KCl electrode. All experiments were performed at room temperature.

The elemental analysis of the polypyrrole films was performed using X-ray photoelectron spectroscopy (XPS). The XPS analysis was performed with a Physical Electronics Quantum 2000 XPS-spectrometer equipped with a monochromatized Al-X-ray source to assess qualitatively the influence of soaking on the composition of these films. The size of the analyzed area was 100 μm in diameter and the analysis depth was about 2–5 nm depending on the investigated element.

The Mathcad 2001 Professional Software (MathSoft, Inc. Canada) was used for the numerical calculations.

2.3. Procedures of PPy–BL–Me electrode preparation

2.3.1. Polypyrrole deposition

The electrodeposition of the polypyrrole films was carried out potentiodynamically from a solution that contained 0.1 M ATP as dopant and 0.1 M pyrrole by potential cycling between 0.0 V and +0.7 V vs. Ag/AgCl/sat.KCl at scan rates of 20 mV s^{-1} (charge density varied from 480 to 960 mC cm^{-2}).

The growth of heparin-doped polypyrrole under potentiostatic or potentiodynamic conditions was performed using solutions containing 16 mg/ml of heparin and 0.1 M pyrrole. The films were prepared under potentiodynamic conditions by cycling the potential between 0.0 V and +0.8 V vs. Ag/AgCl/sat.KCl at scan rates of 20 mV s^{-1}. Potentiostatic growth was achieved by holding a potential at +0.8 V vs. Ag/AgCl/sat.KCl for different times in order to obtain different amounts of charge density (480–840 mC cm^{-2}).

The potentiostatic method was also used to grow polypyrrole films doped with amino acids. PPy–Asn(Gln) films were grown onto the working electrode at a potential of +1.0 V vs. Ag/AgCl/sat.KCl and charge density of 240 mC cm^{-2}. Besides 0.1 M pyrrole, the solution contained 0.1 M Gln (or 0.1 M Asn).

Polypyrrole films doped with sulfosalicylic acid (SSa) were obtained from a solution containing 0.1 M SSa and 0.1 M pyrrole by the potentiostatic method at +0.75 V vs. Ag/AgCl/sat.KCl (the charge density used was 900 mC cm^{-2}).

2.3.2. The process of making calcium and magnesium sensitive PPy–BL films

After synthesis, the polymer membranes were washed with deionized water and then the electrodes were soaked and stored in a mixture of 0.1 M $CaCl_2$ and $Ca(OH)_2$ (pH of about 10.5–11.5) or in saturated $Mg(OH)_2$ solution with a pH of about 10.5. As a rule, a cationic response with a linear range within the Ca^{2+} or Mg^{2+} activities from 10^{-1} M to 10^{-5} M and with a close-to-Nernstian slope was observed for the PPy–BL films after 1 week

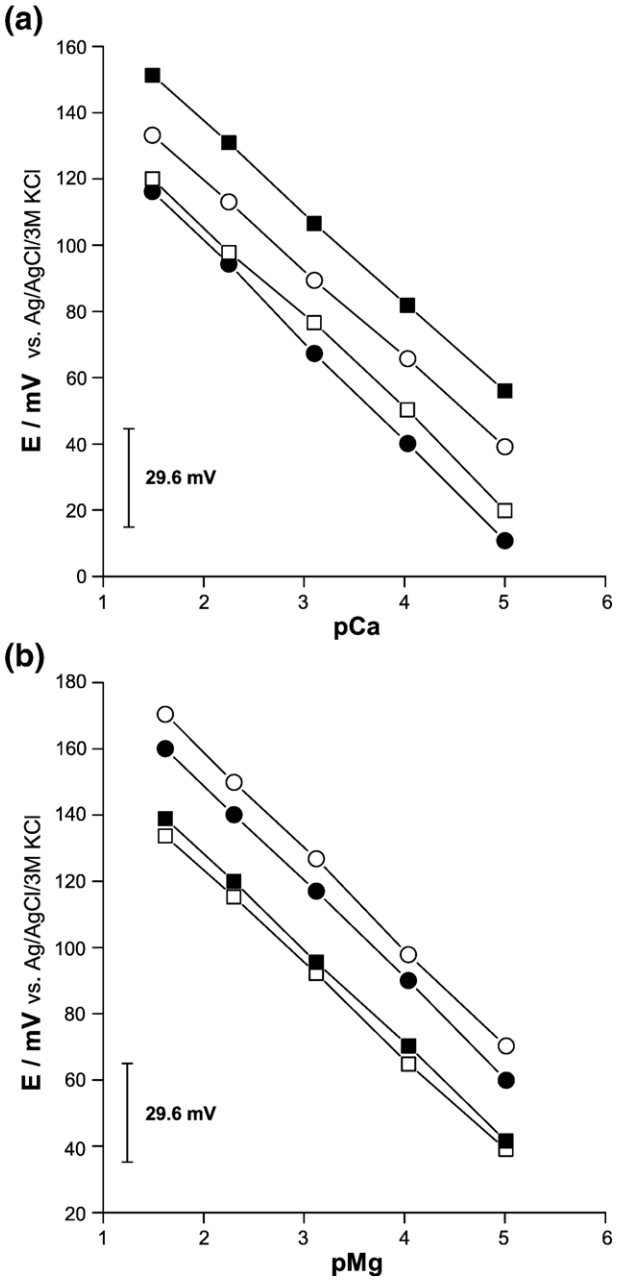

Fig. 2. The Ca (a) and Mg (b) potentiometric sensitivity of polypyrrole films doped with: ATP (open circles) – slope: 28.2 mV/*p*Ca and 29.4 mV/*p*Mg; Hep (full squares) – slope: 27.2 mV/*p*Ca and 28.1 mV/*p*Mg; Asn (open squares) – slope: 26.8 mV/*p*Ca and 29.6 mV/*p*Mg and Gln (full circles) – slope: 30.2 mV/*p*Ca and 28.7 mV/*p*Mg. The calibrations were performed after one month soaking in alkaline solution containing Ca^{2+} or Mg^{2+} ions.

of soaking. As we showed [3], the chemical conditioning of PPy–BL films in the solution of low pH was an ineffective. The cation complexes with BL were formed after PPy–BL film deprotonation in alkaline solutions (protons were substituted with magnesium or calcium cations). Even a long time of post-deposition soaking in an alkaline solution (6 months or longer) any changes in the oxidized state of polymer was observed. On the contrary, conditioning as influencing factor on the film morphological structure may improve the potentiometric response [8].

3. Results and discussion

3.1. Potentiometric response of PPy–BL films

The potentiometric response of the poly(pyrrole) films doped with biological ligands (PPy–BL) was tested in $CaCl_2$ and $MgCl_2$ solutions. The first calibration of the PPy–BL films was always performed without conditioning and no sensitivity towards Ca^{2+} or Mg^{2+} ions was observed, as shown in Fig. 1.

The calcium or magnesium sensitivity was induced by soaking the films in alkaline Ca or Mg solution. After 1 week of conditioning, the PPy–BL–Me films showed a short response time (t_{90} about 10 s), as shown in Fig. 1, for example, for PPy–Asn–Me films. The induced calcium or magnesium sensitivity with a close-to-Nernstian slope in the range of 10^{-1} M to 10^{-5} M Ca^{2+} or Mg^{2+} activities were very stable during a period of 6 months (e.g. 28.2±1.0 mV/pCa and 28.9±0.8 mV/pMg for PPy–Hep–Me films). The representative calibration plots of the PPy–BL films after 1 month of soaking with alkaline Mg or Ca solution are shown in Fig. 2.

3.2. The CP–BL film composition

The chemical composition of the PPy–BL films was investigated by XPS. The presence of the phosphorus peaks in the case of PPy–ATP films (as shown in Fig. 3), and sulfur peaks in the case of PPy–Hep films in the XPS analysis proves

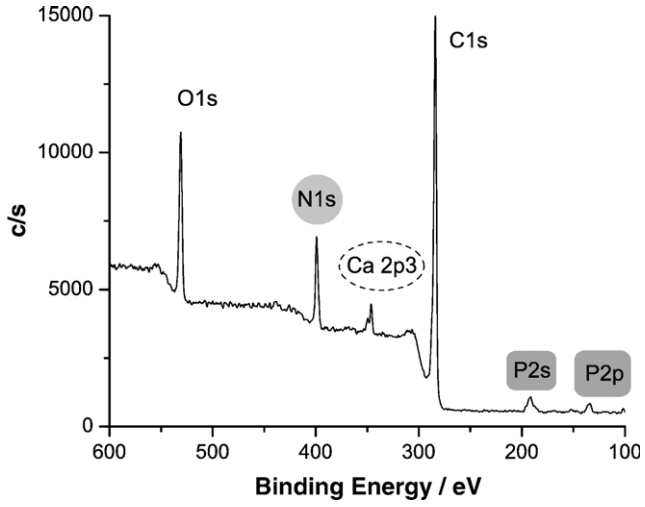

Fig. 3. Exemplary XPS spectrum for PPy–ATP film after conditioning in alkaline calcium solution (pH=10.5–11.5).

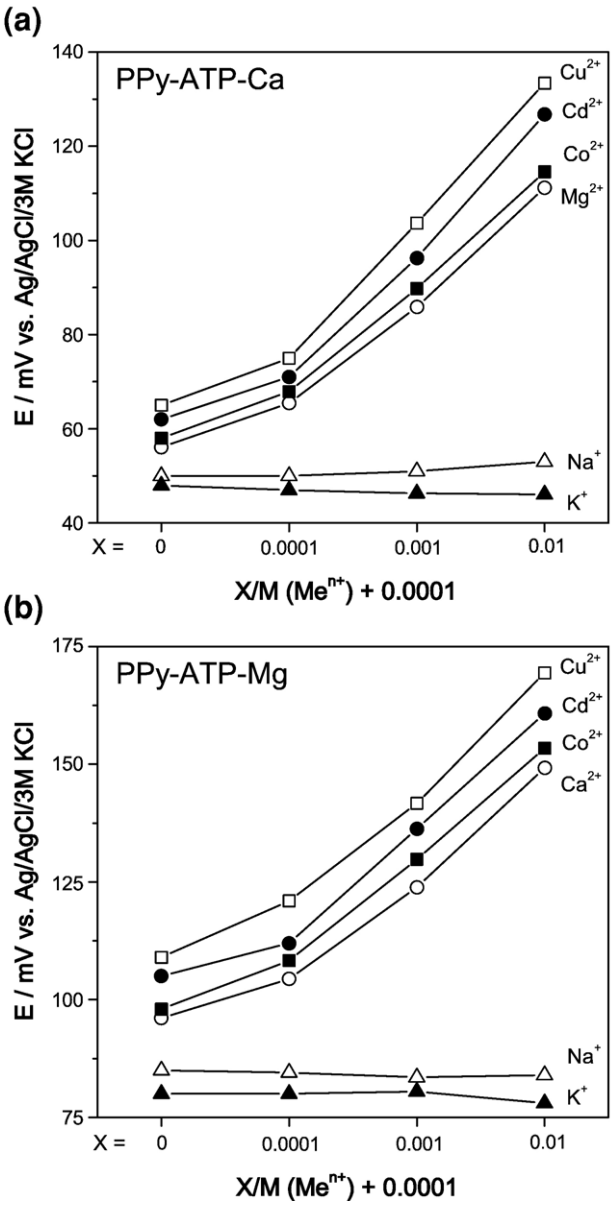

Fig. 4. Potentiometric response of PPy–ATP–Ca (a) and PPy–ATP–Mg (b) membranes for various cations: K^+ (full triangles), Na^+ (open triangles), Mg^{2+} or Ca^{2+} (open circles), Co^{2+} (full squares), Cd^{2+} (full circles), Cu^{2+} (open squares) recorded in mixture solutions of primary and interfering ions (where: (a) [Ca]=0.0001 M, (b) [Mg]=0.0001 M). The measurements were performed for solution buffered with TES (pH=7.4).

that counter ions dope the films formed during electrodeposition. After soaking the PPy–BL films with alkaline Ca or Mg solutions, always additional peaks of calcium (for example, as shown for PPy–ATP film in Fig. 3) or magnesium appeared in the XPS spectrum, thus proving that Ca^{2+} or Mg^{2+} was present in the PPy–BL films.

3.3. Influence of interfering cations on potentiometric response of CP–BL–Me films

It is known that BL can form weak complexes with cations in aqueous solution. For example, in the case of ATP the logarithms

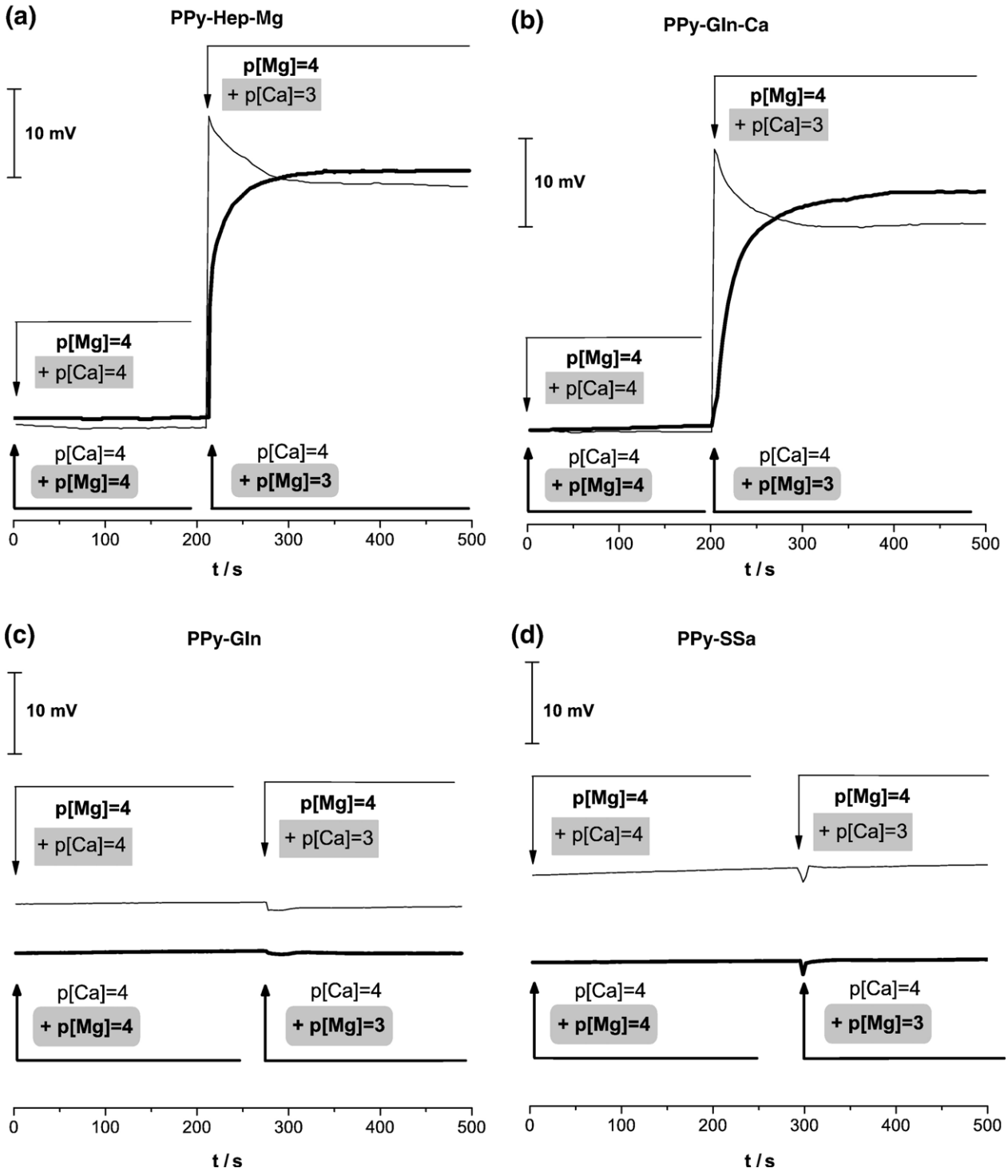

Fig. 5. The typical potential-time behaviour of magnesium and calcium sensitive PPy–BL–Me films (a,b) and insensitive PPy–Gln and PPy–SSa films (c,d) observed after increase a bulk concentration of Ca^{2+} (thin lines) or Mg^{2+} (thick lines) ions (where: $p[X] = -\log([X]/M)$ for X = Ca, Mg).

of stability constants (β_{ATP-Me}) follow the sequence β_{ATP-K} (1.17) ≈ β_{ATP-Na} (1.31) < β_{ATP-Ca} (4.24) ≈ β_{ATP-Mg} (4.55) < β_{ATP-Co} (5.1) < β_{ATP-Cd} (5.68) < β_{ATP-Cu} (6.42) [9]. The stability constant of calcium or magnesium complexes with BL are similar and much bigger than those for sodium or potassium complexes but smaller than those with other divalent cations, the latter belonging to traces in real biological fluids. This observation was used for inducing sensitivity of the PPy–BL films.

As expected, the potentiometric response of the PPy–ATP films towards Na^+ ions was induced after conditioning in NaCl. At the same time, strong interferences of divalent cations were observed. By subsequently soaking the PPy–BL–Na films in the solution of divalent cations (e.g. Ca^{2+}, or Mg^{2+}), forming a

stronger complex with BL, it was possible to obtain calcium or magnesium sensitive films by competitive ion-exchange.

The PPy–BL–Ca(Mg) films were insensitive towards Na^+or K^+ ions, but interferences of other divalent ions were apparent. The potentiometric response of PPy–BL–Ca and PPy–BL–Mg films in solution that first contained only main ions, next also the above mentioned interfering ions, is shown for example in Fig. 4 for polypyrrole film doped with ATP. In order to exclude the pH influence in all solutions during measurements, the pH was buffered by TES to 7.4.

It was also possible to obtain, e.g. copper-sensitive PPy–BL films by soaking the PPy–BL–Na (or Ca, Mg) films in copper solution. As expected, no sensitivity of the PPy–BL–Cu films towards Na^+, Ca^{2+} and Mg^{2+} ion was observed. Since the ATP–Cu complex is the strongest one, it was impossible to reverse the induced copper-sensitivity of the PPy–BL films to sodium, magnesium or calcium ions. This proves that the complexing properties of the BL were retained in the conducting polymer matrix.

3.4. The dynamic response of CP–BL–Me films

In spite of similar sensitivity of both groups of polypyrrole films (namely PPy–BL–Ca and PPy–BL–Mg) towards Ca^{2+} and Mg^{2+} ions, the transitory potential provoked by the changes in bulk concentrations of Ca^{2+} or Mg^{2+} ions was quite different. After an increase of Ca^{2+} bulk concentration in solution, an overshoot-type response was observed; when the Mg^{2+} bulk concentration was increased, a monotonic-type response appeared.

For example, Fig. 5 shows the transitory potential for magnesium sensitive PPy–Hep and calcium sensitive PPy–Gln films caused by Ca^{2+} and Mg^{2+} ions concentration changes. It should be noted that, if the calcium response was checked, the investigated solution contained also 10^{-4} M $MgCl_2$ and vice versa if the magnesium response was checked, then the solution also contained 10^{-4} M $CaCl_2$.

Fig. 5 also shows the potential insensitivity, and lack of transients, of PPy–Gln (before conditioning) and PPy–SSa (SSa cannot form complexes with Ca) films during Ca^{2+} and Mg^{2+} ions concentration changes. These fundamental differences associated with equilibration processes in the Mg–Ca system have already been noticed and discussed for magnesium selective PCV-based electrodes [10].

It is assumed that, for dynamic behaviour of the PPy–BL–Mg and PPy–BL–Ca electrodes, the models previously applied for ion-selective electrodes [11] are valid. When Ca^{2+} or Mg^{2+} interfering ions are added to the bulk solution containing initially the primary ions, both ions participate in competitive binding at the magnesium or calcium sensitive PPy–BL–Me membrane/solution interface. The electrode potential is influenced by the local concentration of ions in the vicinity of the interface, which evolves during equilibration until total equilibrium is attained.

A dynamic response of any p-doped CP (e.g. PPy) film doped with BL is coupled with the ion-exchange process, where Me^{2+} is a primary ion, e.g. Ca^{2+} or Mg^{2+} ion, and N^{2+} interfering ion — Mg^{2+} in the case of CP–BL–Ca electrode and Ca^{2+} in the case of CP–BL–Mg:

$$\overline{\mathrm{CP-BL-Me}} + \mathrm{N}^{2+} \rightleftarrows \overline{\mathrm{CP-BL-N}} + \mathrm{Me}^{2+} \quad (1)$$

The ion-exchange (1) is described by the equilibrium constant $K = \frac{K_{\mathrm{CP-BL-N}} \cdot k_{\mathrm{Me}}}{K_{\mathrm{CP-BL-Me}} \cdot k_{\mathrm{N}}}$, where: $K_{\mathrm{CP-BL-N}}$ and $K_{\mathrm{CP-BL-Me}}$

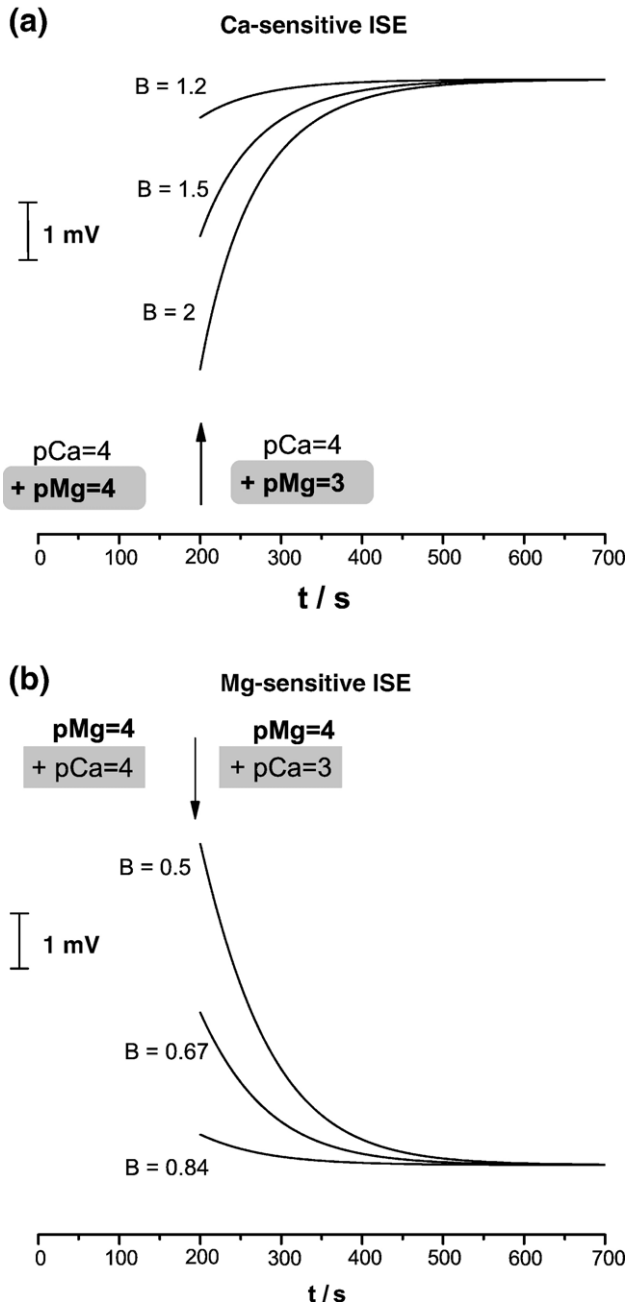

Fig. 6. The time-dependent response of calcium (a) and magnesium (b) sensitive electrode, calculated on the ground DLM model for various B parameter ($B = \frac{U_{\mathrm{N}^{2+}}}{U_{\mathrm{Me}^{2+}}}$ and $K_{\mathrm{Me,N}}=1$): represents response after increase Mg^{2+} activity in the solution of mix magnesium and calcium ions for $\frac{U_{\mathrm{Mg}^{2+}}}{U_{\mathrm{Ca}^{2+}}}=1.2$, 1.5 and 2 potential response after increase Ca^{2+} activity in the solution of mix magnesium and calcium ions for $\frac{U_{\mathrm{Ca}^{2+}}}{U_{\mathrm{Mg}^{2+}}}=0.5$, 0.67 and 0.84.

are stability constants for respective ion complexes in the CP film; k_{Me} and k_N are partition coefficients between bathing solution and CP film for ions Me and N, respectively.

To allow numerical potential-time analysis the concept of the diffusion layer model (DLM) can be adopted (for details see [3]), which has been previously used in the case of ion-selective electrode membranes [11–13].

In principle, the DLM assumes fast and reversible ion-exchange at the membrane sites (local steady state) and linear concentration drops in the vicinity of the ion-exchange sites during equilibration. The DLM as a special case can be reduced to a Nernst–Planck–Poisson model, which has been presented recently for site-based membranes [14–16].

In the DLM, generalized below for any p-doped CP–BL–Me, N film, the electrode potential can be described by the sum of the boundary potential and diffusion potential in the membrane:

$$E = \text{const} + \frac{R\cdot T}{2F}\ln\frac{(1-s_f)\cdot K_{Me,N} + s_f\frac{\bar{U}_{N^{2+}}}{U_{Me^{2+}}}\cdot K_{Me,N}}{K_{Me,N}\cdot(1-s_f)+\frac{D_{N^{2+}}}{D_{Me^{2+}}}\cdot s_f}\frac{\left([Me^{2+}]+\frac{D_{N^{2+}}}{D_{Me^{2+}}}\cdot[N^{2+}]\right)}{\left[\overline{Me^{2+}}\right]_b} \quad (2)$$

The time-dependent function can be expressed by a parameter describing the extent of equilibration (s_f) and is called the

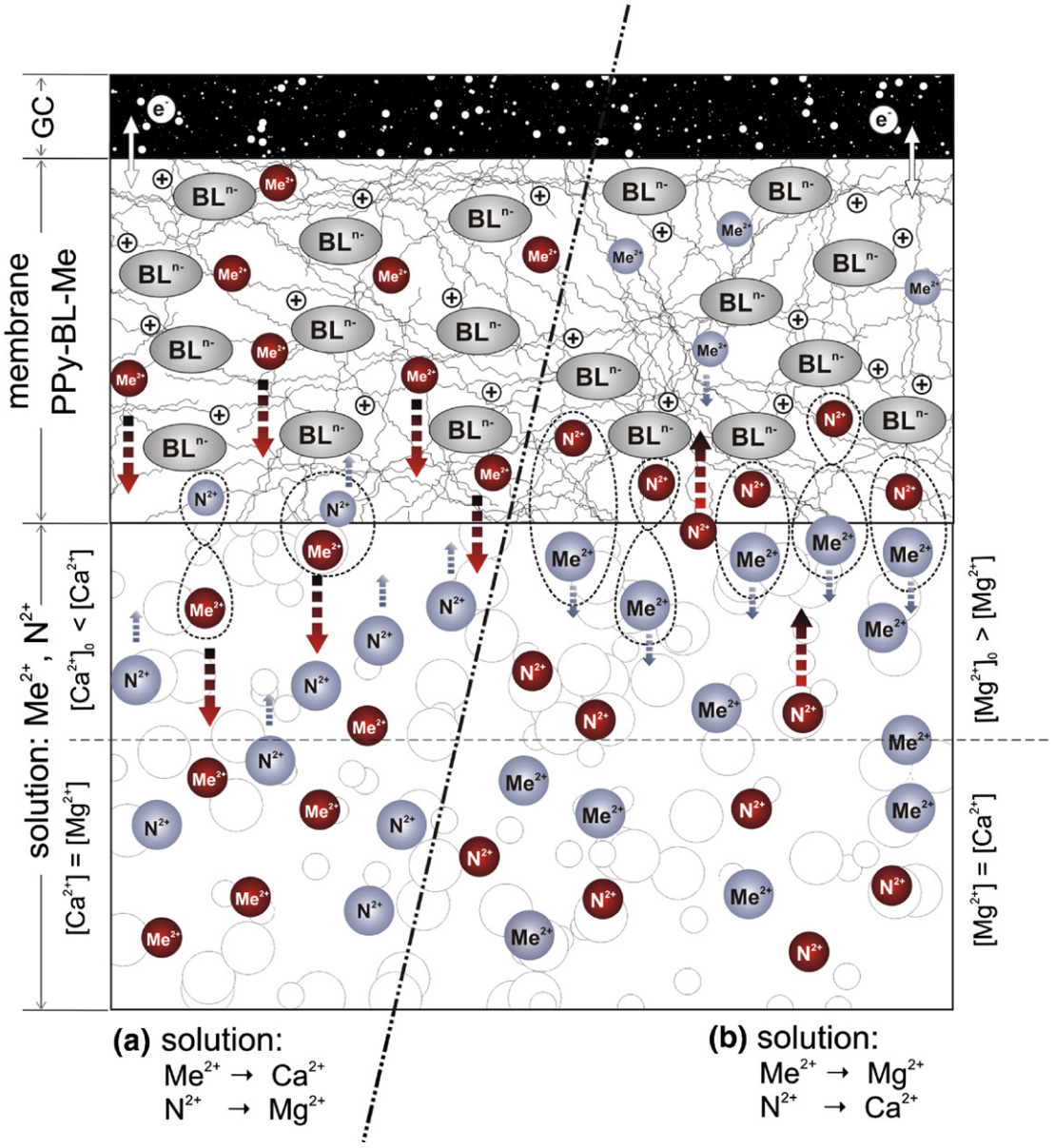

Fig. 7. The ion-exchange processes on PPy–BL–Me electrode in the mixed solution of primary Me^{2+} and interfering N^{2+} ions: a) PPy–BL–Ca – just after interfering ions (Mg^{2+}) concentration change; b) PPy–BL–Mg – just after interfering ions (Ca^{2+}) concentration change. GC – glassy carbon substrate, PPy–BL–Me – calcium (a) or magnesium (b) sensitive poly(pyrrole) film doped with biologically anions.

apparent site-filling (coverage) factor [13]. The function of s_f over time (t) is given by implicit equation:

$$\left[K_{Me,N}-\frac{D_{N^{2+}}}{D_{Me^{2+}}}\right]\cdot s_f - K_{Me,N}\cdot\frac{\left[Me^{2+}\right]+\frac{D_{N^{2+}}}{D_{Me^{2+}}}\cdot\left[N^{2+}\right]}{\left[Me^{2+}\right]+K_{Me,N}\cdot\left[N^{2+}\right]}\ln\left(1-\frac{s_f}{s_{f\,eq}}\right) = \left(\left[Me^{2+}\right]+K_{Me,N}\cdot\left[N^{2+}\right]\right)\cdot C\cdot t \tag{3}$$

with

$$C=\frac{D_{N^{2+}}\cdot A}{n_{tot}\cdot\delta} \tag{4}$$

where: const is a term including contributions independent of the concentration $[Me^{2+}]$ and $[N^{2+}]$; $\left[\overline{Me^{2+}}\right]_b = const'$ and denote ion bulk concentration (M) in the CP film; $D_{Me^{2+}}$ and $D_{N^{2+}}$ are the diffusion coefficients of the primary ion and interfering ion in the aqueous diffusion layer ($dm^2\ s^{-1}$); $\overline{U}_{Me^{2+}}$ and $\overline{U}_{N^{2+}}$ represent the mobilities of ions in the membrane phase; $K_{Me,N}=(K_{CP-BL-Me}/K_{CP-BL-N})K$; $[Me^{2+}]$ and $[N^{2+}]$ are the bulk concentrations of the primary and interfering ions in the solution (after concentration change); A is the electrode surface area (dm^2); n_{tot} is the number of active exchangeable sites occupied by Mg and Ca ions (mol) and t is a time (s); δ is the diffusion layer thickness (dm) and s_f is given by:

$$s_f=\frac{\left[\overline{N^{2+}}\right]_0}{\left[\overline{Me^{2+}}\right]_0+\left[\overline{N^{2+}}\right]_0}=\frac{K_{Me,N}\cdot\left[N^{2+}\right]_0}{\left[Me^{2+}\right]_0+K_{Me,N}\cdot\left[N^{2+}\right]_0} \tag{5}$$

where: $[Me^{2+}]_0$ and $[N^{2+}]_0$ are the ion concentrations at the membrane surface; $\left[\overline{Me^{2+}}\right]_0$ and $\left[\overline{N^{2+}}\right]_0$ represent the ion concentration in the membrane phase at the interface; $\left[\overline{Me^{2+}}\right]_0+\left[\overline{N^{2+}}\right]_0$ is the sum of the Me and N ion concentrations in the surface of the polymer film, and is equal to $\left[\overline{Me^{2+}}\right]_b = \text{const}'$, $s_f=s_{feq}$ when the surface concentrations are equal to the respective bulk concentrations (so-called total equilibrium state) [11].

By coupling Eqs. (2) and (3) it is possible to get the function of potential vs. time and thus a dynamic model of transient effects. In particular, the model allows the prediction of characteristic potential overshoots (formed for changes in bulk concentration of the faster ion – in our case Ca^{2+}), or monotonic changes (caused by changes in bulk concentration of the slower ion – in our case Mg^{2+}) during equilibration, as shown in Fig. 6.

The time-dependent potential profiles observed experimentally are in excellent agreement with these predicted formally. According to the model presented above, the reason for these different potential transients can be sought in slower Mg^{2+} ion transport in comparison to Ca^{2+} and be ascribed to the different hydration energy of these two ions [1–3]. The lower hydration energy of calcium makes the transport of this ion to and into the membrane faster in comparison to magnesium with resulting redistribution of the surface concentration of ions. The influx, or outflow, of slower Mg^{2+} ions determines the speed of the ion-exchange process. This is why, after a change of the Mg^{2+} ion concentration in the solution bulk, deficiency of Ca^{2+} ions in the vicinity of the PPy–BL–Ca film surface vs. bulk is predicted and accordingly a monotonic response type is observed (as shown schematically in Fig. 7(a)). In contrast, after the change of bulk Ca^{2+} ion concentration, the local excess of Mg^{2+} ions at the surface of the PPy–BL–Mg film is predicted and an overshoot-type response is observed (as shown schematically in the Fig. 7(b)).

The physicochemical properties of the cations that participate in competitive ion-exchange processes play a dominant role in distributing these cations in the vicinity of the membrane. The potential-dependent local deficiency or excess of magnesium or calcium ions shown in this paper may affect the speed (i.e. facilitate or retard) of some biological processes.

4. Conclusions

Biological ligands (BLs), such as adenosinotriphosphate, heparin, asparagine and glutamine, which were incorporated during electrochemical deposition into a CP matrix retain their complexing properties known from aqueous solution. This property makes it possible to induce magnesium and calcium sensitivity of CP–BL membranes. After one week of soaking in alkaline solution of calcium or magnesium, the CP–BL films exhibited sensitivity towards these cations with a close-to-Nernstian slope. A different transitory response observed during equilibration induced by a change of bulk magnesium or calcium concentration was ascribed to different rates of magnesium and calcium ion transfer between the bulk of the solution and membrane. It is possible to study the effects of the physicochemical properties of the ions on the potential formation mechanism by using CP–BL–Me films. The same argument applies to real biological membranes. The different mobility of cations engaged in the ion-exchange processes at sites in a real biological system can induce a local redistribution of these ions in their vicinity. This may change the picture of "disposable ions" for membrane potential formation processes, which so far are built assuming validity of bulk intra- and extracellular concentrations of ions.

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