

Dual potentiometric and UV/Vis spectrophotometric disposable sensors with dispersion cast polyaniline

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Abstract Simple conducting polymer–polyaniline-based sensors/biosensors, working either in potentiometric or UV/Vis spectrophotometric mode, are proposed. Disposable sensors were produced by coating polyaniline layers, cast from aqueous dispersion of the polymer nanoparticles, on a transparent plastic polyacetate foil. In the potentiometric mode, polyaniline layers are sensitive to a number of metal cations, while in UV/Vis mode, changes of absorbance were recorded only in case of a chemical reaction of cations with the polymer. Pronounced sensitivity of tested sensors to ammonia was used for potentiometric/spectrophotometric biosensing purposes studied on a model example of urease-based sensors of urea. The highest sensitivity and reproducibility of such sensors were observed in urea concentration ranging from 1 to 10 mM.

Keywords Polyaniline · Aqueous dispersion · Potentiometry · UV/Vis spectrophotometry · Urea biosensor

Introduction

One of the rapidly progressing areas of analytical chemistry is the development of chemical and biochemical sensors. In recent years, particular emphasis is not only on analytical parameters improvement but also on novel sensing materials as well as construction simplification and cost lowering leading to cheap, disposable, and easy to use sensors.

As materials used for sensors construction, particularly promising are conducting polymers [1, 2]. Sensors obtained take advantage of unique properties of these materials related to their conductivity, redox behavior, as well as electrochemical and spectroscopic characteristics. Typical conducting polymers applied polypyrrole, polyaniline (PANI), or poly(3,4-ethylenedioxythiophene) due to their ion-exchange properties, and ability of reversible oxidation/reduction can be used as membranes of electrochemical, potentiometric or amperometric, ion sensors [3–5].

In this group of materials, especially promising is PANI which can undergo both protonation/deprotonation equilibria and multi-step oxidation/reduction associated by color change [6, 7]. This results in both electrochemical properties and UV/Vis spectral changes. However, above-mentioned phenomena in some cases can also result in a quite complicated response pattern, denoting high sensitivity to interferences.

For PANI, either electrochemical or spectrophotometric detection mode has been used, e.g., in pH [8, 9] or ammonia sensors [10–13], as well as in biosensors (e.g., review [14]). Comparison of electrochemical and spectrophotometric mode can bring independent information about influence of various reactants/analytes. However, the same kind of PANI sensor for electrochemical and spectrophotometric modes has been rarely chosen, especially if simplification/miniaturization purposes are additionally required [15].

A significant disadvantage of classical conducting polymers, from the analytical point of view, is the need of electrochemical synthesis and low processability and solubility of resulting material in common solvents. A beneficial solution of this problem is the dispersion of conducting polymers in water or organic solvents, stabilized by appropriate counterions, e.g., aqueous dispersion

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of poly(3,4-ethylenedioxythiophene) (Baytron) or PANI dispersions. Ready-to-use PANI dispersions have been developed by Wessling [16] and now are available commercially. Dispersion cast PANI layers have been recently successfully used as membranes of optical pH sensors [17], in amperometric sensors for ammonia with analytically useful range to concentrations around 10^{-5} M [18], for ascorbic acid [19] or in enzymatic biosensors [20].

The aim of the present work was to develop simple and disposable PANI sensors which can work in dual potentiometric and UV/Vis spectrophotometric detection mode for a similar sensor preparation procedure. Choosing potentiometry as the target electrochemical technique allows using PANI disposable electrodes, which due to applied material are characterized by relatively high resistance. In this group of sensors, applicability of PANI layers cast from nanoparticles dispersion will be checked on example of model analytes as metal cations and ammonia. In sensors construction, the concept of “all-plastic electrodes” will be used [21, 22]. As it has been shown earlier, potentiometric responses of dispersion cast conducting polymer layers can be affected by polymer properties as well as dopant ion or stabilizing substances added to commercial polymer preparation [21]. In “all-plastic” arrangement, the same polyacetate support is suitable for potentiometric and UV/Vis measurement.

For analytical applications, particularly interesting is sensitivity of PANI to hydrogen ions and ammonia, because such species can be also products of enzymatic hydrolysis reactions. This opens an application area for disposable sensors in bioanalytes assessment. This promising possibility will be checked on a model example of urease-based urea optical/potentiometric biosensors [23–25], where PANI layer will be additionally coated by a polymeric (cellulose acetate) layer containing enzyme. The response range and sensitivity of both methods (potentiometric and spectrophotometric) of urea assessment will be compared.

Experimental

Apparatus, electrodes, and chemicals

In the potentiometric experiments, a multi-channel data acquisition setup and software, Lawson Labs. Inc. (3217 Phoenixville Pike, Malvern, PA, 19355, USA), and a double junction Ag/AgCl reference electrode with 1 M lithium acetate in outer sleeve (Möller Glasbläserei, Zürich, Switzerland) were used. In voltammetric and electrochemical impedance measurements, a CH 660A electrochemical workstation (CH Instruments, USA) was used with glassy carbon (GC) electrode of surface area 0.07 cm^2 (polished by Al_2O_3 $0.3\text{ }\mu\text{m}$), platinum counter electrode (surface area,

$\sim 2\text{ cm}^2$), and reference electrode described above. Electrochemical impedance spectroscopy measurements were carried out in the frequency range from 100 kHz to 0.1 Hz, with amplitude 10 mV.

In the UV–Vis spectrophotometric experiments, a diode array spectrophotometer model HP 4852A and conventional $10\times 10\times 45\text{ mm}$ cuvettes No./REF 67.755 (Starstedt, Germany) were used.

Doubly distilled and freshly deionized water (resistance $18.2\text{ M}\Omega\text{ cm}$, Milli-Qplus, Millipore, Austria) was used throughout this work. All used chemicals were of analytical grade and were obtained from POCh (Gliwice, Poland), with the exception of urease, 57 U mg^{-1} (Sigma, Germany) and of PANI. Commercial aqueous dispersion of PANI—D1033W—used for potentiometric and spectrophotometric experiments (conductivity according to producer data 200 S cm^{-1}) was purchased from Ormecon GmbH (Amersbeck, Germany). Some details of this material were described earlier [16, 26]. PANI layers on GC electrodes were obtained by coating the surface with $10\text{ }\mu\text{L}$ of PANI dispersion and drying under ambient conditions.

All-plastic sensors

All-plastic potentiometric sensors were prepared using a procedure similar to that described earlier [21]. A rectangle frame $4\times 23\text{ mm}$ was laser printed on an overhead transparency (Laser/Copier Transparencies Plain, type C, Xerox). This printed pattern was used in order to retain the conducting polymer suspension inside the desired shape, as aqueous dispersion of PANI wets the transparency but stays away from the printed pattern. Sixty microliters of PANI was pipetted to fill the printed shape. After overnight drying of applied conducting polymer dispersion at ambient temperature (resulting in a layer of thickness close to 0.01 mm , measured by micrometer), a strip of PTFE adhesive was applied to isolate the sensor/transducer and the electrical lead part of the CP layer. For biosensors applications, the transducer part was then covered with $15\text{ }\mu\text{L}$ of dispersion containing 19 mg urease and 2% (w/w) of cellulose acetate solution in acetone. After acetone evaporation, the enzymatic sensor was conditioned in 1 mM Tris (pH=7.5) buffer for 20 min.

Polymer layers for UV–Vis spectrophotometric experiments were obtained using PANI dispersion diluted 1:5 with deionized water; $10\text{ }\mu\text{L}$ of this solution was applied on acetate transparency sheets. After overnight drying of applied diluted dispersion at ambient temperature, nearly round spots of diameter 8 mm were obtained. Similarly, as in case of potentiometric biosensors, in optical biosensors, the PANI layer was covered with $15\text{ }\mu\text{L}$ of dispersion containing 19 mg urease and 2% (w/w) of cellulose acetate

solution in acetone. After acetone evaporation, the strips were conditioned in 1 mM Tris buffer (pH=7.5) for 20 min. The foil strips with spots were immersed into absorption cuvette with assay solution and glued (above the solution level) to the wall to locate the spot in solution always on the same height. Then, UV/Vis measurements in the bathing solution were carried out in situ.

In some experiments, the obtained PANI layers (both potentiometric sensors and spots) were deprotonated in freshly prepared saturated $\text{Ca}(\text{OH})_2$ solution in 0.1 M NaCl. Deprotonation time was 5 min in the case of spots and 30 min for plastic electrodes (in the latter case, only the sensor part of the electrode was immersed in the deprotonation solution).

Unless otherwise stated, both potentiometric and spectrophotometric experiments were carried out in electrolyte solutions mentioned in the text, while for purposes of urea determination, the measurements were carried out in 0.1 M NaCl solutions containing Tris buffer (pH=7.5) with addition of urea.

All experiments were performed at temperature of 25 °C.

Results and discussion

Electrochemical studies on glassy carbon electrodes with polyaniline

The introductory electrochemical experiments concerned PANI drop cast on GC electrode support. It should be mentioned that a dispersion cast PANI layer is a less precisely defined system (structure uniformity, thickness, and presence of added stabilizers) compared to electrochemically obtained films. However, the shape of voltammetric curves was similar to those for PANI obtained electrochemically [27] and showed dependence on solution pH (Fig. 1a). In the studied potential range from -0.5 to 0.5 V, typically, a pair of peaks, anodic at potential close to 0.3 V (in acidic solution) and cathodic at 0.1 V, was recorded. In 1 M HNO_3 , these peaks were high and well shaped, with current plateau at potential higher than 0.4 V; however, with pH increase, the peaks became less sharp, and the peak currents decreased. In neutral solution (0.1 M KNO_3), both anodic and cathodic peaks were shifted to lower potentials.

Electrochemical impedance spectroscopy measurements (Fig. 1b) in acidic medium revealed a small semicircle at higher frequencies, pointing to charge transfer resistance not exceeding 20Ω and a capacitive-like behavior at lower frequencies. The low frequency capacitance estimated from this part was in the range of ~ 10 mF, i.e., a value typical for conducting polymers coated on an electrode (e.g., poly(3,4-ethylenedioxythiophene) [28]). In neutral solution (0.1 M

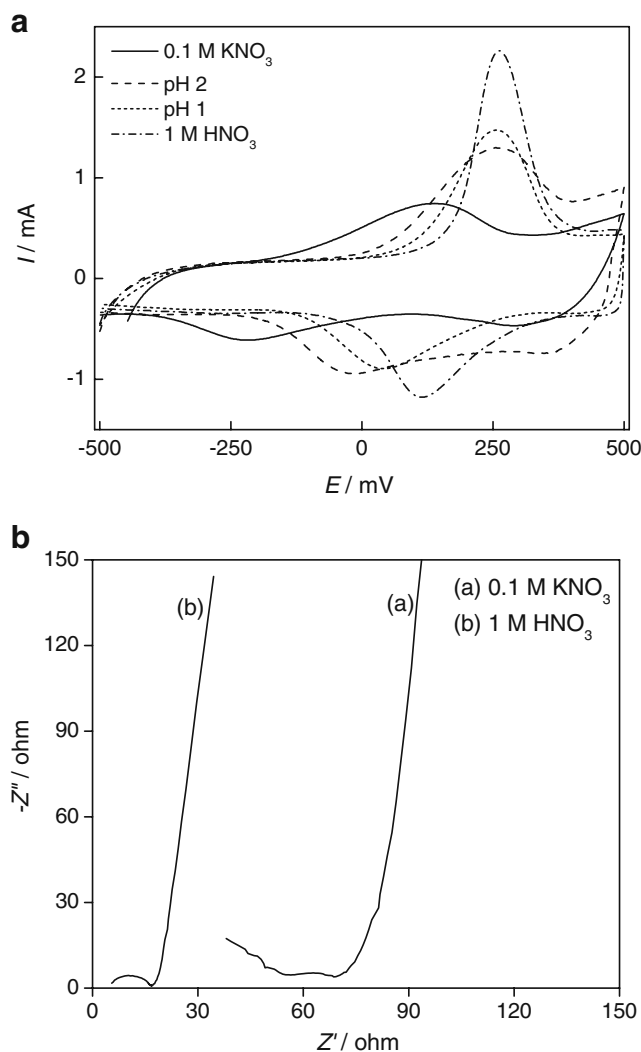


Fig. 1 Cyclic voltammetric curves with scan rate of 50 mV s^{-1} (a) and electrochemical impedance spectra (b) recorded for glassy carbon electrodes coated by polyaniline cast from dispersion

KNO_3), a high frequency deformed semicircle with increased diameter was recorded, pointing to increased charge transfer resistance. For lower frequencies, a Warburg impedance part was visible, indicating a limited ion mobility in the film, and then the spectrum transformed to capacitive like one for the lowest frequencies. The obtained low frequency capacitance was slightly lower than in acidic solution, now equal to 9 mF .

PANI layers can exhibit also potentiometric sensitivity to various species present in the solution. Since it is well known that PANI layers are pH sensitive, the influence of solution acidity on potential of PANI layers coated on GC electrode was tested. pH values were established by addition of HNO_3 or NaOH solutions. Linear dependences with sub-Nernstian slope ($41 \pm 2 \text{ mV pH unit}^{-1}$) were obtained in the acidic pH range, while for $\text{pH} > 6$, the potential was practically independent of pH (Fig. 2a).

However, as known for other polymers, e.g., polypyrrole [4], potential of conducting polymer-coated electrodes can be also sensitive to other ions present in solution. In such a case, linear dependences of potential on logarithm of electrolyte ions concentration/activity have been recorded, with slope depending on ion-exchange properties of the layer. Figure 2b presents potentiometric plots, as dependence of potential on logarithm of electrolyte concentration, recorded for PANI layer in KNO_3 solutions in the concentration, range from 0.1 to 10^{-5} M, shortly after immersion. A linear dependence with sub-Nernstian slope ($38 \pm 1.5 \text{ mV dec}^{-1}$) was obtained, and the positive value of the slope points to cation-exchange properties of these films, resulting from presence of large toluenesulfonate doping ions in the polymer [16].

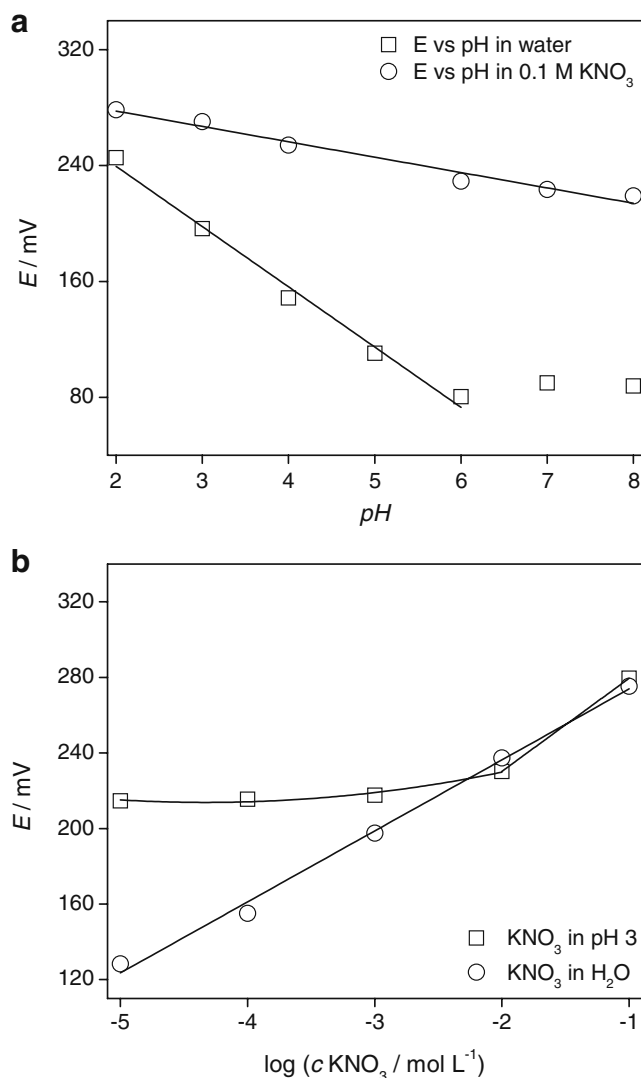


Fig. 2 Dependence of open circuit potential on pH (a) or logarithm of KNO_3 concentration (b) for glassy carbon electrodes coated by polyaniline cast from dispersion

Due to exposed sensitivity to different cations (as shown on example of H^+ and K^+ ions), potentiometric characteristics were also recorded in solutions containing both kinds of cations. In the presence of KNO_3 of constant concentration 0.1 M and for changing pH, the slope of potential dependence on pH in the range from 2 to 8 was much lower than in the absence of KNO_3 (Fig. 2a), equal to $\sim 10 \text{ mV pH unit}^{-1}$. On the other hand, the dependence of potential on logarithm of KNO_3 at constant and low pH (shown for $\text{pH}=3$, Fig. 2b) exhibits linear cationic characteristics with slope similar to that in neutral solution, but only in the concentration range 0.1– 10^{-3} M. For lower concentrations, practically no potential changes were observed. This result confirms influence of total cation (K^+ and H^+) concentration on the potential recorded, i.e., low potentiometric selectivity towards +1 cations.

The low slope of potential vs. pH dependence suggests some kinetic limitations in PANI response. Therefore, the role of duration of the measurement was checked by recording potential of GC electrode with PANI layer consecutively in solutions of $\text{pH}=3$ and $\text{pH}=7$. In acidic solution, the response was almost instantaneous, and the recorded potential was stable, while for $\text{pH}=7$, slow potential decrease (20–30 mV in the range of 30 min) was recorded. Thus, depending on time scale of the experiment, one can observe separately the effect of ion exchange (fast process, responsible for dependence on K^+ concentration) and slower effect of deprotonation (considerably slow in neutral solution).

Potentiometric sensitivity of all-plastic electrodes in salts solutions

As the target “all-plastic” sensors arrangement was used, besides GC electrodes with PANI layers, potentiometric responses were also recorded for PANI layers coated on a polyacetate support. Although resistance of these electrodes is higher than for conducting polymer-modified GC electrodes, it is sufficiently low for potentiometric purposes [29]. For plastic electrodes with protonated form of PANI, potentiometric characteristics were recorded in solutions of exemplary salts (usually chlorides with exception of $\text{Pb}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2$, AgNO_3 , and CuSO_4). For each ion, measurements were carried out for a new electrode; stable potentials were recorded in the range of a few minutes. Similar linear plots of dependence of potential on logarithm of electrolyte concentration were recorded in the concentration range from 1 to 10^{-4} M (Fig. 3), with detection limit within the range 10^{-5} – 10^{-4} M. The obtained plot parameters were collected in Table 1. For most cations, the slopes were close to Nernstian; positive values of slopes for all cations point to cation-exchange properties of the polymer layer containing high amount of large anions, doping the

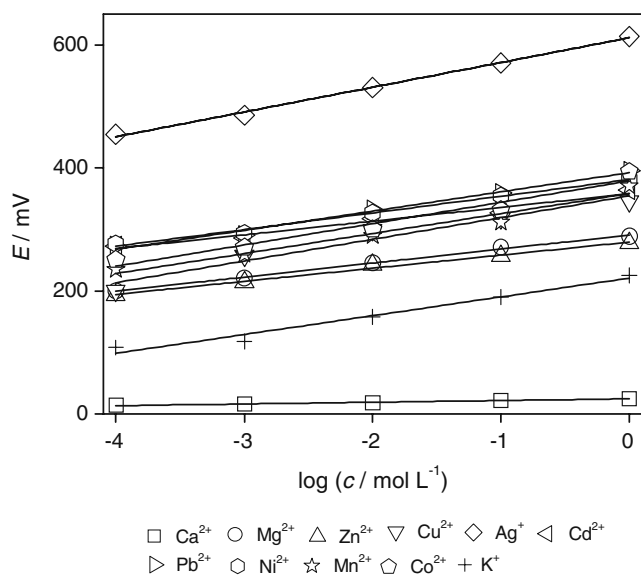


Fig. 3 Potentiometric plots recorded in solutions of different cations for all-plastic PANI electrodes

polymer and used to stabilize the nanoparticles dispersion. On the other hand, some differences in intercept values (E^0) are remarkable showing difference in sensitivity for various cations. This behavior is typical for classical electropolymerized conducting polymer layers, although the plastic sensors are characterized by disposability and simpler preparation method. These results show also that PANI layers exhibit similar sensitivity to some heavy metal cations, while Zn^{2+} and Mg^{2+} ions are practically discriminated (about 0.1 V lower intercept value). Moreover, the potential values for K^+ ions are significantly lower than for heavy metal cations, with slope much lower than Nernstian, pointing to higher sensitivity of polyaniline layers to the latter group of ions.

Important property of an ion sensor is dependence of the signal on history of the electrode and direction of analyte

concentration changes. This effect was checked on example of plastic electrode tested in KNO_3 solution, consecutively, 0.1 and 10^{-3} M. The potential values in both solutions were reproducible (within the range of single millivolt) and stable, only the time needed to obtain stable value in more dilute solution is longer (about 3 min).

However, for several cations, quite different results were obtained. In the case of Ca^{2+} ions, the slope close to zero and significantly lower E^0 value were obtained. This effect results from participation of Ca^{2+} in the membrane cross-linking [30], leading to polymer deprotonation. Such effect is expected also for Mg^{2+} ions [30], where close to Nernstian slope of potentiometric characteristic was observed. However, after longer (1 h) conditioning in 1 M $MgCl_2$ solution, the slope of calibration plot was significantly lower, close to 10 mV dec^{-1} . This confirms lower rate of ion–polymer binding in case of magnesium ions.

On the other hand, in the presence of Ag^+ ions, the intercept was significantly higher than obtained for other cations. This effect can result from reaction between Ag^+ cations and PANI, leading to PANI oxidation. Evidence of such reactions was reported earlier for Ag^+ ions, using mass spectrometry measurements with laser ablation of the polymer, for dispersion cast PANI, showing incorporation of such elements into the PANI layers [22].

A well-documented method of inducing or enhancing cationic sensitivity of conducting polymers to cations is deprotonation process [31]—due to electrostatic reason, release of hydrogen cations would stimulate incorporation of cations, as shown on example of polypyrrole layers. This possibility was also checked in the case of deprotonated PANI. However, the results obtained for deprotonated PANI layers did not differ significantly from those recorded for non-deprotonated layers. This can denote that even for non-deprotonated polymer, due to presence of immobilized large anionic dopant anions, the cation exchange properties

Table 1 Parameters of potentiometric calibration plots recorded for plastic non-deprotonated polyaniline electrodes, in solutions of several analytes within concentration range $1-10^{-4}$ M

Analyte	Slope \pm SD (mVdec^{-1})	Intercept, $E^0 \pm$ SD (mV)	R^2
Zn(II)	22.5 ± 0.7	280 ± 3	0.997
Mg(II)	24.7 ± 0.3	291 ± 2	0.999
Cu(II)	36 ± 4.5	355 ± 10	0.955
Pb(II)	30 ± 2	392 ± 5	0.991
Ni(II)	36 ± 3	381 ± 3	0.977
Mn(II)	36 ± 3	358 ± 3	0.975
Co(II)	34 ± 3	390 ± 10	0.980
Ca(II)	4.6 ± 0.6	25 ± 1	0.945
Cd(II)	23 ± 3	358 ± 6	0.961
Ag(I)	40 ± 1	612 ± 3	0.997
K(I)	31 ± 2	221 ± 7	0.985
NH_3	-49 ± 3	-172 ± 7	0.988
Urea	-30 ± 4^a		0.961

^a In the concentration range $10^{-3} - 10^{-2}$ M

are sufficiently exposed. A significant difference concerns only Pb^{2+} ions, where about 0.1 V higher intercept was obtained, pointing to stronger interactions of Pb^{2+} cations with deprotonated PANI layer or doping ion. On the other hand, for $\text{Ag}(\text{I})$ ions, slightly lower intercept (compared to non-deprotonated layer) was obtained, depending on direction of concentration changes (increasing or decreasing). This effect can relate to lower potential of deprotonated polyaniline, enhancing the reduction process of silver ions. This confirms that the response to Ag^+ ions results from a redox reaction [22], and the final potential after achieving redox equilibrium can be lower than for non-deprotonated layer, also due to presence of metallic silver. Moreover, in the case of Ca^{2+} ions, particularly at higher concentrations, a negative slope was recorded indicating anion exchanging properties. Most probably, after deprotonation in the presence of Ca^{2+} , the polymer contains strongly bound ions which can not participate in cation exchange process, and thus inversion from cation to anion exchange is observed.

In conclusion, PANI layers coated on a plastic support exhibit potentiometric sensitivity to a number of cations, although obtained selectivity is low, limiting the application area of such sensors. However, these results show an advantageous effect that simply coated PANI layers exhibit similar ionic sensitivity as typical electrothesized conducting polymers. Moreover, the response mechanism (e.g., ion exchange or redox reaction) cannot be distinguished basing on potentiometric data; therefore, in the following section, UV/Vis mode was tested for this kind of sensors.

UV/Vis spectroscopy of all-plastic sensors in salts solutions

UV/Vis spectra were recorded in the wavelength range from 330 to 800 nm. In the measurements, both non-deprotonated and deprotonated PANI layers were used. Figure 4 presents spectrum recorded for protonated (emeraldine salt, green) PANI spot (from diluted PANI dispersion). For this polymer layer, an absorption maximum was observed at wavelength close to 770 nm and a broad minimum in the range 480–600 nm. This spectrum is consistent with spectra of protonated PANI obtained from similar dispersions [17]. Analogous spectrum was recorded also for deprotonated polymer (emeraldine base, blue); it shows a characteristic broad peak at wavelength 490–600 nm with a minimum at 400 nm. The spectrum is also dependent on pH of solution, where the spot is immersed; in HCl solution, deprotonated PANI changes its spectrum to typical for protonated polymer.

In the presence of metal cations, generally, one can expect two response mechanisms: (1) simple cation exchange equilibrium or (2) reaction of the polymer with metal ion (e.g., redox reaction) or complexing these cation

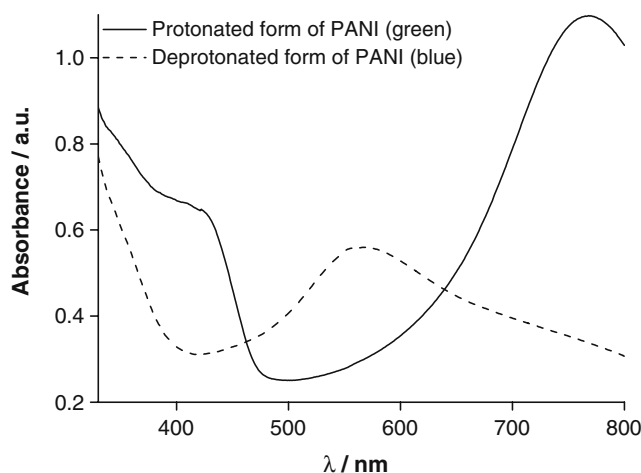


Fig. 4 UV/Vis spectra for protonated and deprotonated form of dispersion cast PANI on a plastic support

and removing cations present in the polymer (H^+ or Ca^{2+} cations for the protonated and deprotonated form, respectively).

In the case of KCl , ZnCl_2 , NiCl_2 , CdCl_2 , CoCl_2 , and MgCl_2 , typical spectra of protonated PANI were recorded, and neither significant nor regular changes in the spectrum were observed pointing to lack of chemical reactions with the polymer. These results suggest simple ion exchange equilibrium between the polymer/dopant and solution, without noticeable change of the polymer composition. It should be underlined that in the potentiometric mode, typical characteristics were recorded for the same cations, with slope close to Nernstian and similar E^0 values (with exception of $\text{Zn}(\text{II})$ and $\text{Mg}(\text{II})$).

On the other hand, in CaCl_2 solutions, more remarkable changes were observed, with absorption decrease at higher wavelengths, e.g., 760 nm. This change (similarly as in potentiometric mode) can be ascribed to interaction of Ca^{2+} with doping anions accompanied by gradual release of H^+ ions, thus the spectrum changes to the form characteristic for deprotonated polymer. Such strong binding of calcium ions with the dopant can be responsible for observed lack of potentiometric sensitivity to Ca^{2+} ions. A significantly smaller absorbance decrease was observed in CuCl_2 solutions. However, because potentiometric sensitivity to copper ions was observed, interaction of Cu^{2+} ions with the polymer dopant is of lower significance.

Another case was represented by $\text{Pb}(\text{NO}_3)_2$ and particularly AgNO_3 solutions, for which significantly high potentials were recorded (see previous section). Now spectral changes were more significant; absorbance increase was observed at higher wavelengths. Particularly, absorbance increase observed at 760 nm suggests oxidation of the polymer film by silver ions with deposition of metallic silver. This redox reaction with silver deposition was earlier

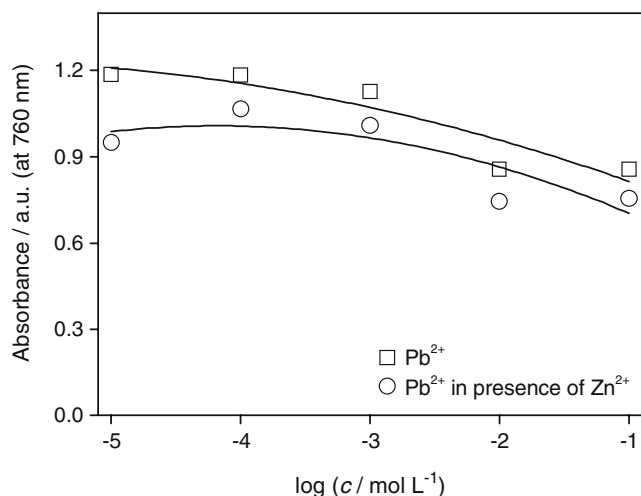


Fig. 5 Dependence of absorbance at 760 nm for PANI spots on plastic support for different Pb^{2+} ions concentration, in the absence or presence of 1 mM $\text{Zn}(\text{NO}_3)_2$

confirmed by experiments using mass spectrometry and cyclic voltammetry [22]. A similar effect, but less exposed, was also observed for $\text{Pb}(\text{II})$ ions, connected probably with accumulation of $\text{Pb}(\text{II})$ ions in the layer. This process should be electrostatically favored for the deprotonated form of the polymer, compared to protonated one. These results are consistent with potentiometric data obtained for $\text{Pb}(\text{II})$ interacting with deprotonated polymer—in that case, the obtained potential values were higher than those for other cations.

In the following experiments, the influence of electrolyte (cation) concentration was tested. Generally, only a small influence of concentration in the range from 0.1 to 10^{-5} M was observed (also for Ca^{2+} cations, where potentiometric insensitivity was observed), with the exception of $\text{Pb}(\text{II})$ where more visible changes of absorbance were observed for the concentration change from 10^{-3} to 0.1 M. In the range of higher wavelengths, a decrease of absorbance (e. g., at 760 nm) was recorded with increasing $\text{Pb}(\text{II})$ concentration (Fig. 5). Although the decrease is low, this result clearly confirms difference in response mechanism for Pb^{2+} ions and other cations. Moreover, the presence of $\text{Zn}(\text{II})$ ions, representing ion-exchange mechanism, does not affect significantly the obtained results. This response differs significantly from that recorded in potentiometric mode, where the difference in signals for $\text{Pb}(\text{II})$ and other cations was hardly visible in measurement carried out for non-deprotonated PANI layers.

Although the spectrophotometric mode does not offer satisfactory sensitivity from analytical point of view, the obtained results are dependent on response mechanism (no spectral changes in case of typical ion exchange and significant changes in case of chemical transformation of the polymer).

Responses of polyaniline layers to ammonia

Ammonia is regarded as an important analyte in different biological, industrial, and environmental samples. Moreover, ammonia can be a product released in enzymatically catalyzed reactions of bioanalytes. Therefore, simple and fast methods of ammonia determination are of interest.

In the first step, the influence of dissolved ammonia on electrochemical properties of PANI layers coated from dispersion on GC electrodes was studied. Figure 6 presents voltammetric curves recorded in ammonia-free supporting electrolyte (0.1 M KNO_3) and in the presence of ammonia. The peaks shape significantly changes with maximum shifted to lower potential and decreasing current at positive potentials, in the presence of ammonia concentration above 1 mM. After re-immersion in NH_3 -free electrolyte solution, the initial shape of the voltammetric curve was almost regained. After following immersion in acidic solution (pH = 3), significant increase of anodic current was observed. This result shows high and reversible influence of ammonia on electroactivity of dispersion-based PANI, similarly as for classical (e.g., electropolymerized) PANI.

The aims of the next experiment were to distinguish the chemical species responsible for observed effects (ammonia or OH^- ions) and the role of basic/acidic form of ammonia (NH_3 or NH_4^+). Voltammetric experiments carried out in 10^{-3} M NaOH (the same concentration of OH^- ions as in 0.1 M NH_3) did not show significant changes in the recorded current (compared to the curve recorded in supporting electrolyte only). On the other hand, experiments carried out for increasing NH_4NO_3 concentration did not reveal significant changes. Moreover, experiments conducted in buffer solutions ($\text{NH}_3 + \text{NH}_4\text{NO}_3$) showed similar behavior as in the presence of NH_3 of the same

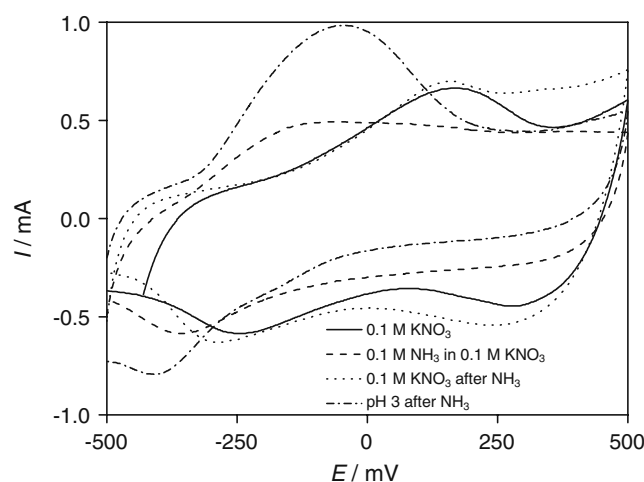


Fig. 6 Cyclic voltammetric curves recorded for glassy carbon electrodes coated by polyaniline cast from dispersion in the absence or presence of ammonia, scan rate of 50 mV s^{-1}

concentration as NH_3 in the buffer, although the pH value was significantly lower compared to solution of NH_3 . These results confirm that, under applied conditions, solely NH_3 affects electrochemical properties of PANI.

The next set of experiments was carried out for plastic sensors (non-deprotonated PANI coated on a transparent plastic support), carried out both in potentiometric and spectrophotometric mode. Under potentiometric conditions, addition of 10^{-4} M ammonia resulted in significant potential decrease, almost 0.1 V; however, a stable response was obtained after about 15 min. This potential decrease can be ascribed to slow polymer deprotonation. With increasing analyte concentration, the response time was shorter, e.g., for 0.01 M ammonia, it was in the range of 2 min. Figure 7 shows dependence of the potential on logarithm of ammonia concentration; for each concentration, the measurement was carried out on a freshly prepared layer. For all sensors, the initial potential of the plastic electrode was similar. The final potential decreased with increasing NH_3 concentration, and two sections for such dependence can be distinguished: significant changes in the range 10^{-4} to 10^{-3} M with potential drop around 200 mV dec^{-1} and the range of linear dependence at higher concentrations from 10^{-3} M to 1 M with Nernstian slope $-61 \pm 7 \text{ mV dec}^{-1}$. The high sensitivity (super-Nernstian) in the low concentration range can result from occurring qualitative transformation of the polymer from protonated to deprotonated form, with rate highly dependent on analyte concentration. On the other hand, for higher ammonia concentrations, smaller potential decrease concerns some changes in deprotonation state, without qualitative change of the polymer properties. Although the reproducibility for one sensor was poor, particularly for lower ammonia

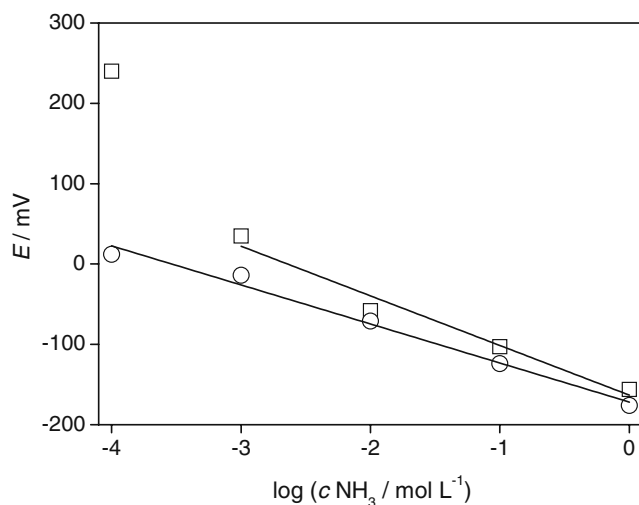


Fig. 7 Dependence of potential of all-plastic PANI electrodes on logarithm of ammonia concentration: each point for a separate sensor (squares); points for the same sensor and decreasing concentration (circles)

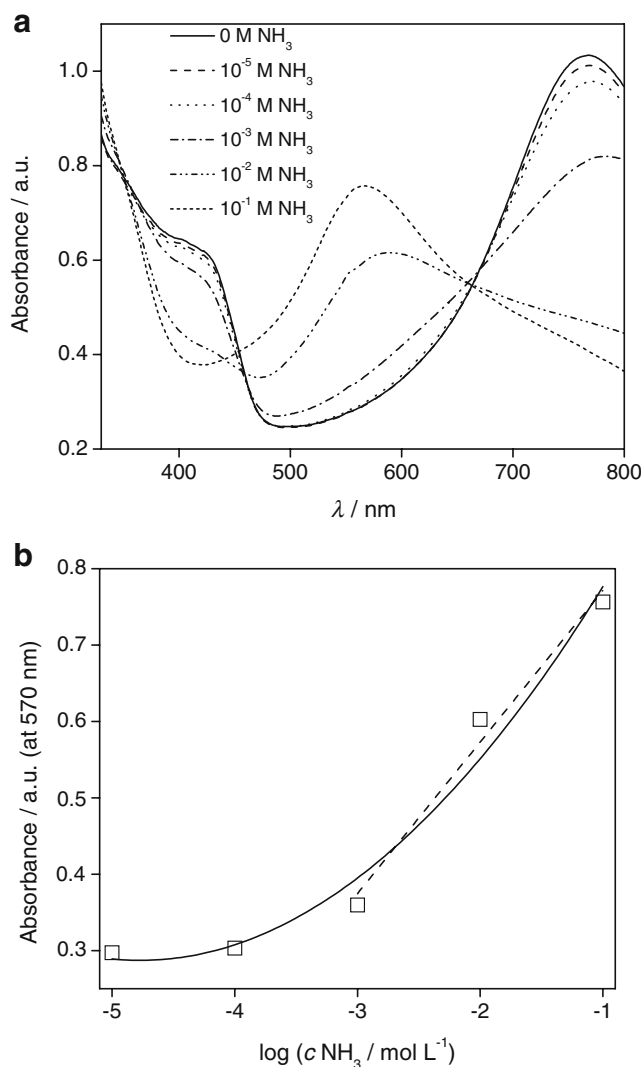


Fig. 8 UV/Vis spectra of dispersion cast PANI on plastic support for various concentrations of ammonia (a); dependence of absorbance on ammonia concentration at wavelength 570 nm (b)

concentrations, due to polymer transformation, the reproducibility for a group of sensors in the same solution within a single millivolt was recorded. These results confirm that plastic electrodes can practically serve as disposable sensors only, of good reproducibility and high sensitivity to ammonia, particularly for concentrations lower than 0.01 M. Therefore, taking into account the significantly high sensitivity in the range from 10^{-4} to 0.01 M, such electrodes can particularly serve as simple potentiometric ammonia detectors, when a level inside this concentration range is surpassed.

The above assumption concerning two states of the sensing polymer layer in different concentration ranges of NH_3 seems to be confirmed by calibration plot recorded for a sequence of decreasing ammonia concentration changes recorded for the same layer. In such a case, a linear plot was

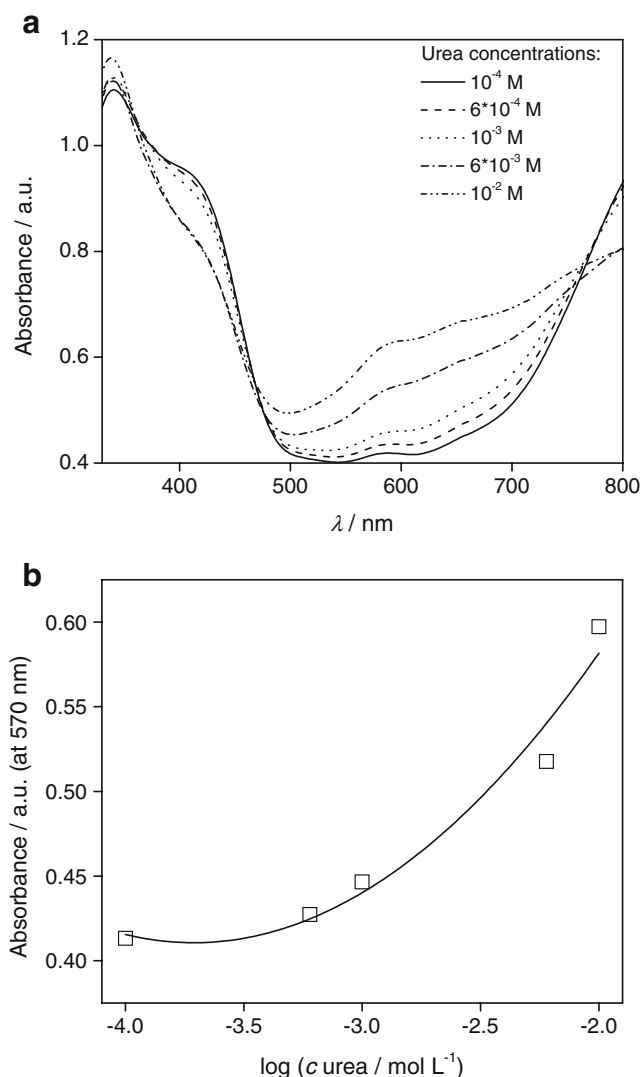


Fig. 9 UV/Vis spectra of dispersion cast PANI with urease containing layer on plastic support for various concentrations of urea (a); dependence of absorbance on urea concentration at wavelength 570 nm (b)

obtained in the entire concentration range (for concentration from 1 M to 10^{-4} M, Fig. 7), with slope lower than Nernstian ($-49 \pm 3 \text{ mV dec}^{-1}$, Table 1). During measurement in the most concentrated solution, the layer became deprotonated to a significant extent, thus the protonation/deprotonation state did not change significantly for lower ammonia concentrations. Therefore, preliminary deprotonated PANI layers are better candidates for potentiometric ammonia sensors operating in a wider linear range.

UV/Vis measurements were carried out also for non-deprotonated PANI spots in the concentration range from 0.1 to 10^{-5} M. UV/Vis spectra obtained for several ammonia concentrations are presented in Fig. 8a. For concentration up to 10^{-3} M, spectra typical for protonated PANI in the wavelength range from 480 to 600 nm, but

lower absorbance (compared to ammonia-free solution) beyond this region were recorded. This confirms gradual change from protonated to deprotonated state for ammonia concentration below 10^{-3} M. On the other hand, for NH_3 concentration 0.01 or 0.1 M, spectra typical for deprotonated PANI layer were observed. These results are consistent with potentiometric data confirming two concentration ranges with different sensitivity of responses: slow transformation from protonated to deprotonated form for NH_3 concentration not higher than 1 mM and fast changes corresponding to final deprotonated state for concentration above 10^{-3} M. For wavelength 570 nm, where absorbance changes upon ammonia addition are large, a plot of absorbance vs. logarithm of ammonia concentration was shown (Fig. 8b). Two concentration ranges of ammonia, mentioned above, are reflected in the shape of the plot: linear dependence of absorbance on logarithm of ammonia concentration for concentration higher than 10^{-3} M, with slope $0.20 \pm 0.02 \text{ absorbance unit dec}^{-1}$, and lower changes for concentration below 10^{-3} M. Comparing resolution of potentiometric and spectrophotometric method (1 mV and 0.01 absorbance unit, respectively) and relating them to slopes of experimental linear dependences, the former method seems approximately three times more sensitive to ammonia concentration changes than UV/Vis spectrophotometry; moreover, it is linear (in relation to logarithm of ammonia concentration) in a wider concentration range.

Disposable urea biosensors

PANI layers coated from nanoparticles dispersion prevailing sensitivity to ammonia at low concentrations can be utilized in simple disposable biosensors, where NH_3 is

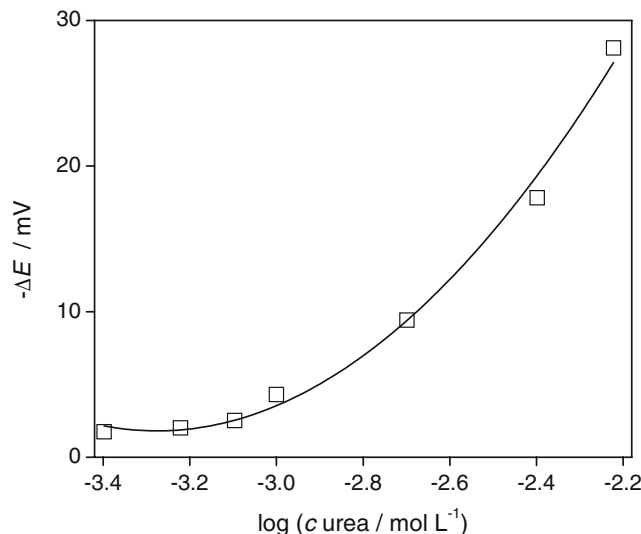


Fig. 10 Dependence of potential changes (related to urea-free buffer solution) of all-plastic PANI electrodes on logarithm of urea concentration

released in the enzymatic reaction. Such possibility was studied on example of a model urea enzymatic biosensor. The idea of urea biosensors was to simply cover the PANI spot by a cellulose polyacetate membrane with incorporated enzyme—urease. In the presence of urea, enzymatically driven reaction of ammonia release should occur, and locally formed ammonia is expected to deprotonate the polymer, resulting in analytical signal changes of either UV/Vis spectrum (Fig. 9a) or open circuit potential. Such changes were recorded in solution with supporting electrolyte (0.1 M NaCl) and 1 mM Tris buffer of pH=7.5.

The response time resulting from enzymatic reaction was in the range of minutes. Hence, in the following measurements, the signal (absorbance) was read after 180 s. Figure 9b shows dependence of absorbance at wavelength 570 nm for various concentrations of urea. With growing reactant concentration, transformation of the spectrum to typical for deprotonated PANI can be observed. This confirms that the detection limit of urea, using this method, is below 1 mM. The highest sensitivity of this method (slope close to 0.1 absorbance unit dec^{-1}) observed in the urea concentration range ca. 1–10 mM (Fig. 9b) is beneficial from the practical point of view, as it corresponds to physiological range of urea concentration in human blood serum. Taking into account the resolution of UV/Vis spectrophotometric method as 0.01, the distinguishable concentration changes of urea correspond to 0.1 log unit.

For this system, also potentiometric experiments were carried out. Upon addition of urea in the concentration range from 0.5 to 10 mM, significant potential decrease was recorded. These changes were reversible; after re-immersion of the sensor in urea-free buffer solution, the initial potential value was regained. The dependence of potential on logarithm of urea concentration was linear with slope -30 mV dec^{-1} for urea concentration above 1 mM (Table 1 and Fig. 10). Taking into account the resolution of potentiometric measurement equal to 1 mV, the distinguishable concentration changes of urea is 0.03 log units, i.e., three times smaller than in the case of UV/Vis spectrophotometry.

Conclusions

Owing to their electrochemical and optical properties, PANI layers cast from nanoparticles dispersion on a plastic transparent support can be used as simple and disposable potentiometric or UV/Vis spectrophotometric sensors. Their applicability was tested for several analytes: metal cations, ammonia, as well as urea undergoing enzymatic hydrolysis to ammonia. Although in potentiometric mode PANI is sensitive towards numerous cations, its analytical applicability is limited due to low selectivity.

In the spectrophotometric mode, the response mechanism (ion exchange, chemical reaction of the polymer) can be distinguished, which is typically obscured under potentiometric conditions.

Promising analytical results concern applicability of PANI sensors for ammonia detection in concentration range from 10^{-4} to 1 M, particularly in potentiometric mode. Taking into account high sensitivity to ammonia at low concentrations, PANI layers can be applied in urea biosensors utilizing enzymatically driven hydrolysis in the presence of immobilized urease, applicable in the physiological range of urea concentration in blood serum. The results revealed also higher sensitivity of potentiometric mode compared to spectrophotometric method, both to ammonia and urea concentration changes.

The main advantage of the proposed sensors results from their simplicity, ease of preparation, and working in both potentiometric and spectrophotometric mode.

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