

Full Research Paper

## Optimalization of Poly(neutral red) Coated-wire Electrode for Determination of Citrate in Soft Drinks

Gabriela Broncová\*, Tatiana V. Shishkanova, Martin Krondak, Radko Volf and Vladimír Král

Department of Analytical Chemistry, Institute of Chemical Technology, Technická 5, 166 28 Prague 6 Czech Republic

\*Author to whom correspondence should be addressed. E-mail: Gabriela.Broncova@vscht.cz

Received: 9 October 2007 / Accepted: 28 January 2008 / Published: 4 February 2007

---

**Abstract:** This report presents an optimization of potentiometric measurements with citrate-selective electropolymerized poly(neutral red) electrodes. The optimal background electrolyte for these measurements is a TRIS buffer with nitrate at pH 8.5. The electrodes described here exhibit stable and reproducible near-Nernstian response to citrates with a low detection limit of  $6 \times 10^{-6}$  M. Electrodes polymerized from sulfuric acid and acetonitrile are compared in detail. Simple and sensitive method for quantification of citrate in real-life samples by potentiometry with poly(neutral red) electrodes are presented. Data from potentiometric measurements of citrate are compared with capillary electrophoresis.

**Keywords:** conductive polymer, poly(neutral red), coated-wire electrode, citrate, real-life sample.

---

### 1. Introduction

Conductive polymers (CPs) are a promising group of compounds, which are widely applied in chemical sensors and a variety of other applications [1,2]. One type of sensors is of noble metal electrodes coated with a conductive polymer film. These electrodes, when used for potentiometric measurements, are called coated-wire electrodes (CWE). Electropolymerizations of pyrrole, aniline or thiophene are often used for the preparation of such sensors. The polymer layer is relatively easy to prepare and results in thick and homogenous coverage of the metal. Unfortunately, the above-

mentioned films are not selective [3]. To create a selective CP-based receptor requires a polymerizable and selective monomer that is also stable under the polymerization conditions.

The CP's selectivity is controlled by doping the film from solution or by incorporating a recognition unit into the conjugated polymeric backbone. In terms of potentiometry, the film acts as a cation or anion exchanger and provides a cationic or anionic potentiometric response [4,5]. Additionally, electron transfer across the polymer–solution phase boundary affects the potentiometric response in the presence of a redox-active species [5,6]. Sensors based on conductive polymers have distinct advantages, such as simple preparation, fast response towards the analyte and potential for miniaturization [7]. Their lifetime is longer than classical PVC membrane electrodes [7,8], because there is no receptor (ionophore) loss from the membrane due to the low solubility of the polymeric film in conventional organic and inorganic solvents [9]. However there are still very few CP-based electrodes for the determination of anions. To date, both inorganic ( $\text{NO}_3^-$ ,  $\text{ClO}_4^-$ ,  $\text{SCN}^-$ ,  $\text{Cl}^-$ ) and organic (dodecylsulfate, dodecylbenzene sulfonate, ascorbate) anions have been determined [10], but most of the published data was acquired using calibration solutions instead of real-life samples to avoid matrix effects. Anion analysis in real samples (washing-up liquid and washing powder) was only performed with dodecyl benzene sulfonate ( $\text{DBS}^-$ )-doped polyaniline [11].

Citric acid is often used to inhibit the growth of microorganisms in food [12]. This acid is used as a preservative additive in non-alcoholic beverages at concentrations ranging from  $10^{-3}$  to  $10^{-1}$  M. The standard methods for citric acid determination are described in review [13]. Electrochemical [14], spectrophotometric [12], chromatographic [15] and enzymatic [16] methods are employed. A copper-selective tubular electrode was used to determine citrate for flow injection with potentiometric detection in juices [14]. The copper ions in solution react with citrates and this electrode determines the residual copper concentration. A selective PVC electrode based on a zirconium(IV)-tetraphenylporphyrin complex has been described, including its application in citrate determination in juices [17]. The results were compared with RP-HPLC, but the performance parameters of ISE (lifetime, signal stability and response time) are missing. To date, no electrochemically prepared polymer has the ability to interact with citrates.

Recently, our group has found that electropolymerized films obtained from neutral red (NR) showed a high sensitivity and selectivity for citrate among the various carboxylates [18]. The selectivity of poly(neutral red) films (PNR) towards citrate was affected by their preparation conditions, namely, polymerization from either aqueous or organic solution. Preliminary potentiometric measurements indicated the possibility of using PNR electrodes in the analytical analysis of soft drinks. However, such problems as the measurement protocol for optimal PNR performance, the reproducibility of the preparation of PNR electrodes, and long-term drifts in the potentiometric measurements remain unresolved. Therefore, the present publication resolves *i*) the optimization of the experimental conditions for potentiometric citrate determination (i.e. choice of suitable buffer and salt to maintain the optimal pH and ionic strength of the sample in question), *ii*) the stabilization of the potentiometric signal by conditioning (in terms of its dynamic response and long-term stability). In addition, the variability of the citrate potentiometric determinations and its correlation with the homogeneity of the polymer coating is discussed.

## 2. Materials and Methods

### 2.1. Reagents

3-Amino-7-dimethylamino-2-methylfenazine=Neutral red (NR), all inorganic salts, HCl, H<sub>2</sub>SO<sub>4</sub> and NaOH were obtained from Lachema and Penta (Czech Republic). Tetrabutylammonium perchlorate (TBAP), Tetradecyltrimethylammonium bromide (TDTABr), Tris(hydroxymethyl)aminomethane hydrochloride and Tris(hydroxymethyl)-aminomethane (TRIS), 2-(N-Morpholino)ethanesulfonic acid hydrate (MES), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) and acetonitrile were obtained from Sigma Aldrich (Germany). Working solutions for potentiometric measurements were prepared from redistilled water. Deionised Milli-Q water (Millipore, Bedford, MA, USA) was used for capillary electrophoresis. The soft drink samples given in table 1 originated from local stores of Tesco and Delvita (Czech Republic).

**Table 1.** Composition of the soft drink samples.

Sample	Composition of soft drinks
Gatorade - orange	Water, glucose syrup, saccharose, citric acid, sodium citrate, NaCl, KH <sub>2</sub> PO <sub>4</sub> , emulsifier E414 and E445, MgCO <sub>3</sub> , aroma, dyes E104, E110
Hello juice - apple	Drinking water, sugar, apple juice, apple aroma, citric acid, preservatives E202 and E211, dyes E104 and E133
Joe's baby cola	Drinking water, sugar, phosphoric acid, citric acid, E211, natural aroma, dye caramel, caffeine, CO <sub>2</sub>
Kofola	Water, syrup KOFO (syrup, sugar, water, caramel, dye E150d, citric acid, NaCl, herbal extract, licorice extract, E211, caffeine), CO <sub>2</sub>
Mountain Dew - lemon	Water, sugar, citric acid, E211, caffeine, stabilizer E414 and E445, sodium citrate, aroma, ascorbic acid, dye E102 (tartrazine yellow), CO <sub>2</sub>
Poděbradka - lemon	Mineral water (HCO <sub>3</sub> <sup>-</sup> , Cl <sup>-</sup> , Na <sup>+</sup> , Ca <sup>2+</sup> ), sugar, citric acid, natural aromatic compounds, E211, CO <sub>2</sub>
Zón - lemon	Drinking water; sugar, citric acid, ascorbic acid, E202, E211, CO <sub>2</sub> .

Dyes: E102 (tartrazine yellow); E104 (quinoline yellow); E110 (yellow SY); E 133 (brilliant blue); E150d (ammonia-sulfite caramel); preservatives: E202 (potassium sorbate); E211 (sodium benzoate); emulsifiers and stabilizers: E414 (Arabic gum); E445 (glycerolester of pine resin).

### 2.2. Electrode preparation

Electropolymerized films of NR were prepared by anodic oxidation in a cyclic voltammetric regime in aqueous (3 M H<sub>2</sub>SO<sub>4</sub>, PNR(aq)) and organic medium (acetonitrile with 0.05 M TBAP, PNR(org)). Detailed descriptions of the preparation procedures can be found in [18].

### 2.3. Potentiometric measurements

Potentiometric measurements were performed using an ISE tester [19] vs. Ag/AgCl electrode at ambient temperature. The pH was monitored using a type SEUJ 212 glass electrode (Turnov, Czech Republic) in conjunction with a type PHI 04 MG pH-Meter (Labió, Czech Republic).

Sufficient aqueous solution of salt was employed to maintain constant ionic strength. The salts were chosen from the following set: Na<sub>2</sub>SO<sub>4</sub>, NaNO<sub>3</sub>, NaCl, NaH<sub>2</sub>PO<sub>4</sub>, NaClO<sub>4</sub>, NaSCN and NaI at pH 6.5. The influences of each of the pH buffers (HEPES, MES, TRIS) on potentiometric response were examined over a concentration range of 10<sup>-5</sup> – 10<sup>-1</sup> M. The solution pH was adjusted by adding H<sub>2</sub>SO<sub>4</sub> for HEPES and MES and HCl for TRIS. Solid NaOH was used to increase pH.

Due to the protonation processes of citric acid, the potentiometric response was measured under acidic (pH 3 and 5) and alkaline (pH 8.5 and 11) conditions. These pHs were maintained using MES (0.05 M) and TRIS (0.02 M).

After optimization, the performance parameters of PNR electrodes were measured (calibration plot curve, working range, practical detection limit (PDL) and hysteresis) together with repeatability for 7 electrodes of each kind (PNR(aq) and PNR(org)). The time constant of the PNR electrodes was measured for three sodium citrate concentrations (10<sup>-3</sup>, 10<sup>-2</sup> and 10<sup>-1</sup> M). Electrodes conditioned in water or 10<sup>-3</sup> M sodium citrate were compared.

Long-term stability was monitored by repeated measurements of sodium citrate in the range 10<sup>-3</sup> - 10<sup>-1</sup> M for two months. Electrodes were conditioned in a 10<sup>-3</sup> M citrate solution and stored in distilled water when not in use.

### 2.4. Potentiometric determination of citrate in soft drinks

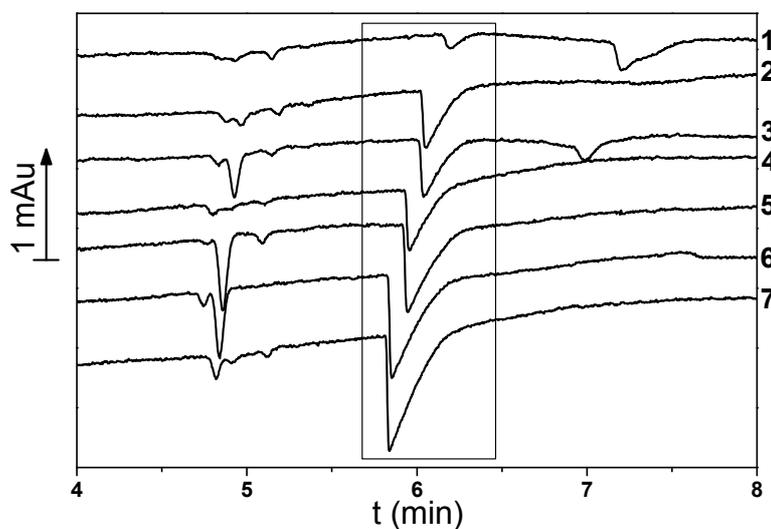
New electrodes were prepared for the measurement of citrate in soft drinks. The freshly prepared electrodes were left in distilled water for 11 days to mature the film. They were then calibrated using a calibration solution of 10<sup>-6</sup> to 10<sup>-1</sup> M sodium citrate in TRIS (0.02 M) and NaNO<sub>3</sub> (0.1 M) at pH 8.5. Beverage samples were stirred for 30 minutes to remove most of their CO<sub>2</sub>. They were subsequently tenfold diluted by the addition of a pH 8.5 solution of TRIS and NaNO<sub>3</sub>. After that, the pH was readjusted to 8.5 by adding solid NaOH. The known addition method [20] was applied by adding 50 µl 0.1 M sodium citrate (pH 8.5 again) twice. Subsequent additions were always double the amount of the previous addition, i.e. 100 µl and 200 µl. Every determination was carried out using three replicate electrodes and every single measurement was repeated. The electrodes were immersed in distilled water for 30 minutes after measuring each sample to regenerate their surface.

### 2.5. Determination of citrate content of soft drinks by capillary electrophoresis

Electrophoresis experiments were performed in an untreated fused-silica capillary of length 39 cm (30 cm to the detector) and 75 µm ID purchased from Composite Metal Service (The Chase Hallow, Worcester, USA). A CAPEL 105 electrophoresis system (Lumex, Saint Petersburg, Russia) was used with indirect UV detection (254 nm). The base electrolyte was 5 mM K<sub>2</sub>CrO<sub>4</sub> with 0.5 mM TDTABr at pH 8.0. All samples and standards were filtered through a disc filter with 0,45 µm pores (Millex HV, Bedford, USA). The capillary was conditioned by sequential washing with 0.1 M HCl (5 min), water

(5 min), 0.1 M NaOH (5 min) and water again (5 min) before measurements began. A five-minute washing with the background electrolyte was done between each measurement. The conditioning was repeated after every beverage sample because of deposition of the sample on the capillary wall.

The samples were injected into the capillary hydrodynamically (1.5 kPa/30 s) and the analyses were run at reverse polarity, -10 kV, at 20 °C for 8 min. The instrument was first calibrated using standards of citrate in water at concentrations from 0.01 mM to 0.2 mM. The peak citrate concentration was registered at around the 6<sup>th</sup> minute. The 7 soft drinks were then analyzed. The samples were diluted 50- or 100-fold before analysis. Figure 1 shows electrophoregrams of the samples. 6 analyses were carried out of each standard and sample. The citrate contents were calculated from the peak areas.

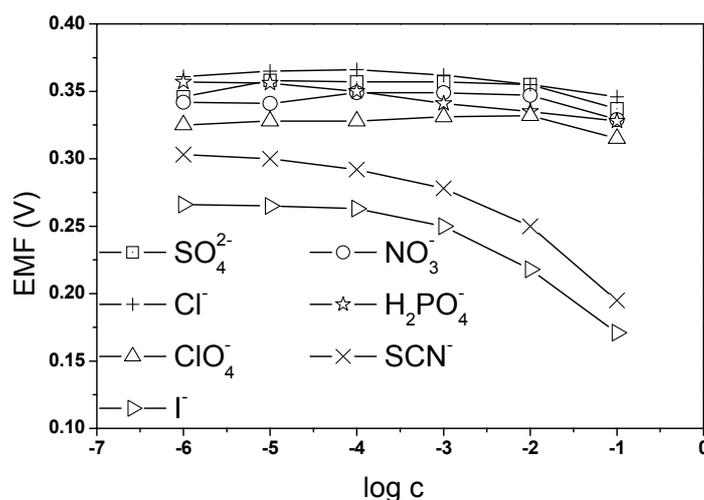


**Figure 1.** Citrate electrophoregrams of soft drinks. The citrate peak is marked by a rectangle; 1 – Joe’s cola, 2 – Mountain Dew, 3 – Kofola, 4 – Zón, 5 – Poděbradka, 6 – Gatorade, 7 – Hello.

### 3. Results and Discussion

#### 3.1. The choice of appropriate medium for response testing with poly(neutral red) electrodes

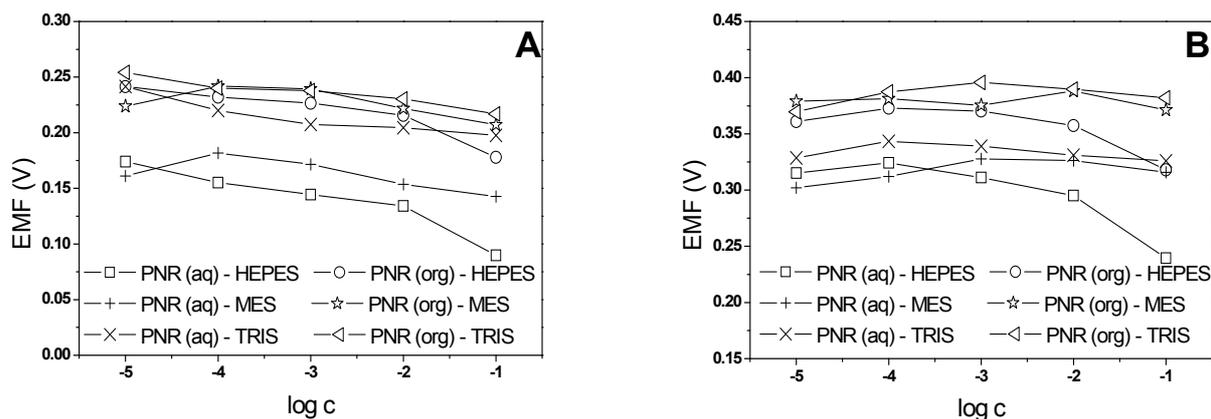
The choice of appropriate environment is essential for measurements with sensitive electropolymerized electrodes [18]. First, the PNR electrodes were tested with inorganic ions in distilled water to choose the ion that best maintained constant ionic strength. The lipophilicity of the selected anions decreases in the sequence  $\text{SCN}^- > \text{I}^- > \text{NO}_3^- > \text{Cl}^- > \text{SO}_4^{2-} > \text{H}_2\text{PO}_4^-$  [21]. PNR electrodes prepared from both an aqueous and organic solution of neutral red gave practically no response to any of the hydrophilic anions (Fig. 2). The electrodes are only sensitive to high concentrations of lipophilic ions such as  $\text{SCN}^-$  and  $\text{I}^-$ . As a result, the chlorides and nitrates of alkali metals are suitable for adjusting ionic strength, because they do not affect the signal of the PNR electrode.



**Figure 2.** Dependence of PNR electrode potential on concentration of inorganic anions.

PNR electrode signals are not affected by a change in the concentration of buffer (HEPES, MES and TRIS) at pH 5 and 8.5. The only exception is a high concentration ( $10^{-2}$  a  $10^{-1}$  M) of the most lipophilic, HEPES (Fig. 3). The potentiometric response of the polymer film toward HEPES is a result of nonselective interactions between the PNR film and buffer at the polymer-sample phase boundary. Both MES and TRIS can be used for measurements with PNR electrodes.

Our preliminary results showed that PNR electrodes are sensitive to citrate at alkaline pH [18]. Therefore the best candidate for potentiometric measurement is TRIS due to its optimal buffer capacity and lower lipophilicity compared to MES.



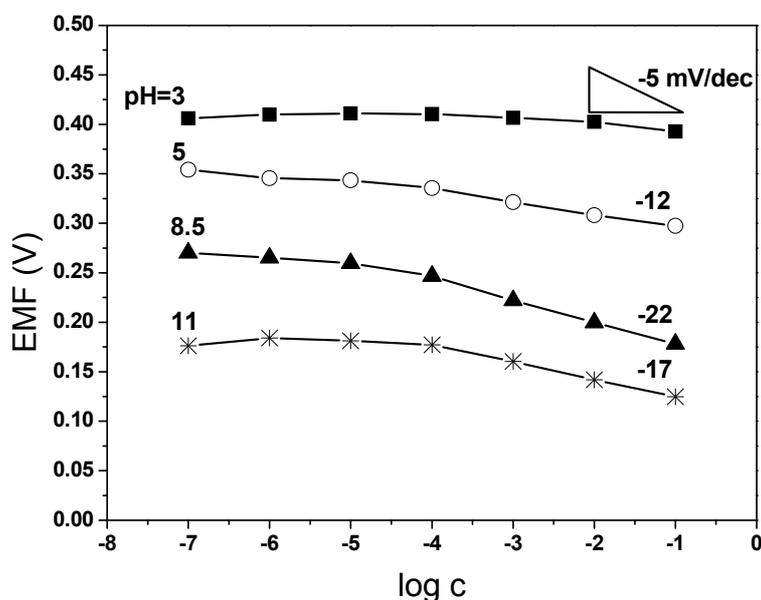
**Figure 3.** Potential dependence of PNR(aq) and PNR(org) electrodes on concentration of buffer (HEPES, MES and TRIS) at pH 8.5 (A) and pH 5 (B).

Citric acid can lose three hydrogen ions ( $pK_1=3.13$ :  $H_3A \leftrightarrow H_2A^- + H^+$  ;  $pK_2=4.75$ :  $H_2A^- \leftrightarrow HA^{2-} + H^+$  ;  $pK_3=6.40$ :  $HA^{2-} \leftrightarrow A^{3-} + H^+$ ) and each of these anions might interact with PNR in a different way. This possibility was examined by measuring the potentiometric response toward citrates at different pHs, in this case 3, 5, 8.5 and 11. Figure 4 shows that the response of the electrode

toward citrate differs with pH. As can be seen from the potentiometric measurements with the PNR electrode, the polymer does not respond to the monovalent ion  $\text{H}_2\text{A}^-$  (pH=3). Only very limited response was observed for the divalent ion  $\text{HA}^{2-}$  (pH=5). The trivalent ion  $\text{A}^{3-}$  (pH=8.5 and 11), on the other hand, gives a nice response with a slope between -22 and -17 mV per concentration decade, which is close to the theoretical -20 mV for the trivalent anion. pH 8.5 is a better choice because the working range is slightly narrower at pH 11.

Similar electrode behavior, including the sensitivity decrease and lowering of detection limit, is the result of protonation/deprotonation processes of both the analyte and receptor. This was also documented by potentiometric measurements of salicylate using metaloporphyrine-based ISEs [22,23].

In conclusion, the previous results show that the best choice of background electrolyte is a mixed solution of 0.02 M TRIS at pH 8.5 and 0.1 M sodium nitrate as an indifferent electrolyte. The most important advantages of this electrolyte, compared with water as the medium, is a pH high enough to deprotonate the citrate anion and a constant ionic strength.



**Figure 4.** Potential response of PNR electrode prepared from aqueous solution toward citrate at various pH's (3 and 5 (MES), 8.5 and 11 (TRIS)).

### 3.2. The characteristic parameters of poly(neutral red) electrodes

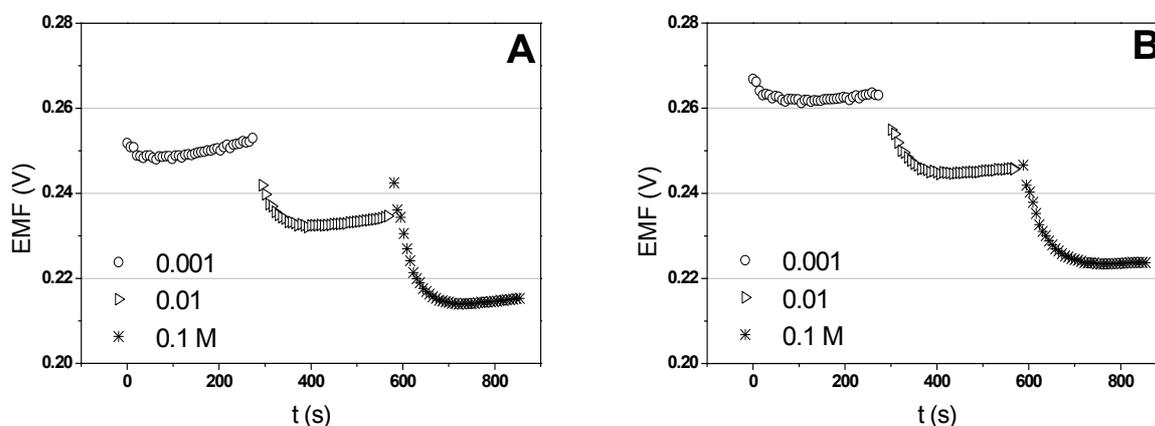
4 or 5 of every seven prepared electrodes provided a Nernstian response over a suitably large linearity range for subsequent measurement. Approximately 35% of the electrodes were rejected. The rest of electrodes gave a Nernstian slope for trivalent ions over the concentration range  $10^{-5}$  -  $10^{-1}$  M. The determination coefficient of regression  $R^2$  was better than 0.985. Both kinds of electrodes (made from aqueous and organic medium) have a relatively low practical detection limit (Table 2).

**Table 2.** Comparison of parameters of PNR electrodes made from aqueous (aq) and organic (org) solution.

Electrode	PNR(aq)	PNR(org)
Slope $S$ (mV decade <sup>-1</sup> )	$-18.7 \pm 3.0$	$-20.1 \pm 2.2$
Potential constant $K$ (mV)	$244.6 \pm 25.6$	$238.8 \pm 12.4$
PDL (M)	$(2 - 6) \times 10^{-6}$	$(2 - 5) \times 10^{-6}$

The electrodes prepared from an organic solution of neutral red have less scattered responses (the deviation is lower) compared with electrodes prepared from aqueous solution. The most probable explanation for this fact is that the surface of the platinum wire was not 100% covered by the PNR film when polymerization was from water. Films prepared from organic solutions are always more compact [24].

The dynamic behavior of the electrodes was tested at three different concentrations. Figure 5 shows the response of electrodes conditioned in citrate solution and conditioned in water. The time to reach the final potential is up to 35 seconds. The response time is the same for both conditioning in citrate or in water, but the potential of PNR electrodes conditioned in citrate was more stable compared to the potential of those soaked in water. Conditioned electrodes responded to citrate anions practically immediately after immersion.

**Figure 5.** Speed of response of PNR electrode prepared from aqueous solution. (A) Electrode conditioned in water (B) electrode conditioned in 0.001 M citrate.

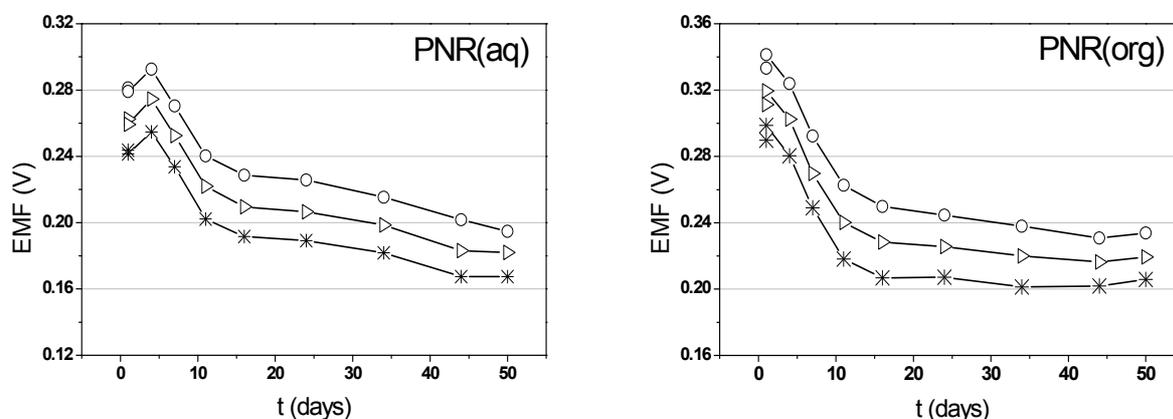
The time constant  $\tau_{95}$  was in the range 10 – 35 s and was proportional to the concentration of the sample. The same behavior was observed for electrodes prepared from organic solution. Similar behavior was described for electrodes based on metalated tetraphenylporphyrine (Co(*o*-NH<sub>2</sub>)TTP) with a  $\tau_{95}$  lower than 25 s for SCN<sup>-</sup> [7].

During an evaluation of hysteresis it was observed that electrodes prepared from acetonitrile exhibit worse hysteresis than electrodes prepared from an aqueous solution. As a result, the slope of the calibration curve was 7 mV per decade lower for measurements starting at the highest concentration than for a calibration curve measured from the lowest concentration. The biggest difference between the potentials measured up and down was in the range 10<sup>-5</sup> - 10<sup>-3</sup> M. The slope changed by about 2 mV

per decade with electrodes prepared from aqueous solution. The potentials only differed at high concentrations of  $10^{-3}$  -  $10^{-2}$  M. The low concentrations were hysteresis-free.

Similar behavior was observed with PEDOT polymer electrodes prepared from acetonitrile, which exhibited considerable hysteresis in low concentrations of  $\text{AgNO}_3$  solution. The hysteresis decreased after pretreatment of the support electrode by cyclic voltammetry before depositing the polymer on the electrode surface [25]. The worse hysteresis of organic PNR electrodes is induced by a non-equilibrium process at the phase boundary that depends on the doping of the polymers. The doping depends on the electrochemical preparation conditions and history of the polymers [26, 27]. Moreover, the nature of the doping anion plays a major role in the electrochemical behavior of the polymers [28]. A memory effect was also observed for polyaniline films doped with perchlorate ions. In contrast, if the polyaniline contained sulphate ions as the dopant, the hysteresis was negligible [27].

During long-term stability experiments, the electrodes had only a low drift, except for the first few measurements, where the potential drifted several tens of millivolts (Fig. 6). The potential stability of the polymeric films is often determined by changes in the composition of the polymeric film after preparation. The oligomers that were incorporated into the polymeric film are gradually washed out during the conditioning of electrodes [28]. In addition, the polymer rearranges its structure on the support metal during conditioning [25]. On the 11<sup>th</sup> day the potential became stable, although a small drift was observed. Nevertheless, the electrode parameters of PNR electrodes such as their slope and concentration range remain unchanged during this period. The drift can be effectively eliminated by calibrating the ISE just before measuring the sample [29].



**Figure 6.** Potential dependence of both types of PNR electrodes on the age of the electrode for three citrate concentrations: 0.001 (○), 0.01 (▷) and 0.1 M (\*).

The calibration curve slope remained unaltered over one and a half months. After this period, a moderate decrease in the slope of about 2-3 mV per concentration decade was observed (Fig. 6). Similar behavior is common to most polymeric electrodes, e.g. a PANI-based electrode for  $\text{DBS}^-$  determination worked for 2 months without a loss of sensitivity. In this case the electrode was stored under an argon atmosphere, because of the sensitivity of PANI to oxygen [11].

A comparison of the electrodes prepared from organic and aqueous medium showed that the initial change in potential is lower for electrodes prepared from aqueous solution. On the other hand, these electrodes have a higher drift after this initial period ( $0.6 - 1.0 \text{ mV day}^{-1}$ ) compared with electrodes

from organic solution ( $0.1 - 0.4 \text{ mV day}^{-1}$ ). Drift is always dependent on the citrate concentration and the lowest drift is with the most concentrated solution.

### 3.3. Determination of citrate content of soft drinks with poly(neutral red) electrodes by potentiometric and electrophoresis methods

The preliminary results demonstrated the possible application of these electrodes in real-life sample analysis [18], because the results obtained were in agreement with those from spectroscopy [30]. The parameters of PNR electrodes, i.e. the almost theoretical slope, wide working range and sufficient selectivity ( $\log K^{\text{pot}}_{\text{Citrate, J}} \leq -0.26$ , where J are various carboxylic acids [18]), allow them to also be used for citrate determination in other real samples.

The polymeric electrodes selected for determining citrate had calibration curves that were linear over the range  $10^{-4} - 10^{-1} \text{ M}$  at least, and their slopes over this range were  $-18.7 \pm 3.0 \text{ mV per decade}$  for PNR(aq) and  $-20.1 \pm 2.2 \text{ mV per decade}$  for PNR(org). These parameters should be enough for the analysis of any sample.

The complicated matrix of samples – which contain various colorants and emulsifiers – resulted in variances for the values obtained of between 7 and 23% (Table 3). This slightly higher variability might be the result of the sorption of sugars and colorants to the electrode surface and consequent modification of the binding properties of the PNR film. The comparison of electrodes prepared from different environments demonstrates that the PNR polymerized from an organic environment has a lower variance, as was mentioned above.

**Table 3.** Determination of citrate content in  $\text{g L}^{-1}$  by Known addition method [20] and by capillary electrophoresis (CE) in soft drinks.

Sample	PNR(aq) electrodes	PNR(org) electrodes	CE
Gatorade - orange	$3.26 \pm 0.76$	$3.01 \pm 0.25$	$3.43 \pm 0.19$
Hello juice - apple	$4.77 \pm 0.78$	$5.09 \pm 0.55$	$3.93 \pm 0.25$
Joe's baby cola	$0.48 \pm 0.06$	$0.40 \pm 0.04$	$0.38 \pm 0.02$
Kofola	$1.22 \pm 0.15$	$1.45 \pm 0.11$	$1.51 \pm 0.11$
Mountain Dew - lemon	$1.13 \pm 0.23$	$1.74 \pm 0.27$	$1.37 \pm 0.08$
Poděbradka - lemon	$2.61 \pm 0.40$	$2.50 \pm 0.23$	$2.82 \pm 0.15$
Zón - lemon	$1.56 \pm 0.25$	$1.37 \pm 0.15$	$1.60 \pm 0.11$

(Mean values of six determinations with confidence interval ( $st\sqrt{n}$ ) at the 95% level.)

On the other hand, the charge of determined ion governs the accuracy in the potentiometric analysis as well. The accuracy of the potentiometric determination is higher for the mono- and divalent ions. To date, the quantity of citrate in beverages is variable about 8 - 10% in individual set of bottles. The determination of citrate (trivalent ion) with R.S.D. value 7% is very optimistic result obtained with coated-wire electrode.

The comparative analysis was performed using capillary electrophoresis with indirect UV detection, which is usually used for the analysis of carboxylic acids in alcoholic [31] and non-alcoholic [32,33] beverages. Capillary electrophoresis is more accurate (its variance is below 7%), but takes twice as

long as potentiometry. The high citrate content in Hello juice obtained by the PNR electrode is a result of strong matrix effect. This is due to the presence of a high amount of colorant (brilliant blue) and malate, which can interfere at high concentrations ( $\log K^{\text{pot}}_{\text{Citrate, Malate}} = -0.59 \pm 0.10$  [18]). Potentiometric analysis is fast and simple. It is not necessary to carry out a complex procedure and also a minimal amount of sample is consumed. Last but not least, the analyses are very cheap.

#### 4. Conclusion

The properties of PNR prepared under various conditions were described together with a description of a citrate ion-selective sensor. The appropriate background electrolyte for potentiometric experiments is a mixed solution of 0.02 M TRIS with 0.1 M sodium nitrate at pH 8.5. The PNR electrodes described here have a low time constant, low hysteresis and provide a relatively stable Nernstian response. Their initial conditioning takes 11 days and then the properties of the electrodes do not change. These results demonstrate the feasibility of using electrodes for screening and rapid determination of citrates in soft drinks. Electrodes prepared from organic solutions exhibit a better potential stability than electrodes prepared from water, because the polymer generates a more compact coverage of the support substrate. In contrast, aqueous electrodes are more accurate and exhibit less hysteresis. The citrate content measured potentiometrically by PNR electrodes was in good agreement with data from capillary electrophoresis.

#### Acknowledgement

Financial support from the Ministry of Education of the Czech Republic Grant No. MSM 6046137307 is gratefully acknowledged.

#### References and Notes

1. Trojanowicz, M. Application of conducting polymers in chemical analysis. *Microchimica Acta* **2003**, *143*, 75-91.
2. Skotheim, T. A.; Elsenbaumer, R. L.; Reynolds, J. R. *Handbook of Conducting Polymers*, Second Edition; Marcel Dekker: New York, 1998.
3. Cadogan, A.; Lewenstam, A.; Ivaska, A. Anionic responses of electrochemically synthesized polypyrrole film. *Talanta* **1992**, *39*(6), 617-620.
4. Bobacka, J.; Ivaska, A.; Lewenstam, A. Potentiometric Ion Sensor Based on Conducting Polymers. *Electroanalysis* **2003**, *15*(5-6), 366-374.
5. Migdalski, J.; Blaz, T.; Lewenstam, A. Electrochemical deposition and properties of polypyrrole films doped with calcion ligands. *Anal. Chim. Acta* **1999**, *395*, 35.
6. Maksymiuk, K.; Nybäck, A.-S.; Bobacka, J.; Ivaska, A.; Lewenstam, A. Metallic and non-metallic redox response of CPs. *J. Electroanal. Chem.* **1997**, *430*, 243-252.
7. Daunert, S.; Wallace, S.; Florido, A.; Bachas, L. G. Anion-selective electrodes based on electropolymerized porphyrin films. *Anal. Chem.* **1991**, *63*(17), 1676-1679.
8. Blair, T. L.; Allen, J. R.; Daunert, S.; Bachas, L. G. Potentiometric and fiber optic sensors for pH based on an electropolymerized cobalt porphyrin. *Anal. Chem.* **1993**, *65*, 2155-2158.

9. Dong, S.; Sun, Z.; Lu, Z. Chloride chemical sensor based on an organic conducting polypyrrole polymer. *Analyst* **1988**, *113*, 1525-1528.
10. Bobacka, J. Conducting Polymer-Based Solid-State Ion-Selective Electrodes. *Electroanalysis* **2006**, *18*(1), 7-18.
11. Karami, H.; Mousavi, M. F. Dodecyl benzene sulfonate anion-selective electrode based on polyaniline-coated electrode. *Talanta* **2004**, *63*, 743-749.
12. Luque-Peréz, E.; Ríos, A.; Valcárcel, M. Flow-injection spectrophotometric determination of citric acid in beverages based on a photochemical reaction. *Anal. Chim. Acta* **1998**, *366*, 231-240.
13. Moreno-Cid, A.; Yebra, M. C.; Santos, X. Flow injection determinations of citric acid: a review. *Talanta* **2004**, *63*, 509-514.
14. Lima, J. L. F. C.; Delerue-Matos, C.; Vaz, M. C. V. F.; Silva, J. Determination of citric acid in soft drinks using flow injection with potentiometric detection. *Fresenius J. Anal. Chem.* **1999**, *364*, 266-269.
15. Morales, M. L.; Ferreira, R.; González, A. G.; Troncoso, A. M. Simultaneous determination of organic acids and sweeteners in soft drinks by ion-exclusion HPLC. *J. Sep. Sci.* **2001**, *24*, 879-884.
16. Matsumo, K.; Tsukatani, T. Simultaneous quantitation of citrate and isocitrate in citrus juice by a flow-injection method based on the use of enzyme reactors. *Anal. Chim. Acta* **1996**, *321*, 157-164.
17. Hattori, H.; Hoshino, M.; Wakii, T.; Yuchi, A. Effects of Two-Phase Reactions on Composition and Potential Response of Zirconium(IV)-Tetraphenylporphyrin Complexes as Carrier for Citrate-Selective Electrode. *Anal. Chem.* **2004**, *76*, 5056-5062.
18. Broncová, G.; Shishkanova, T. V.; Matějka, P.; Volf, R.; Král, V. Citrate selectivity of poly(neutral red) electropolymerized films. *Anal. Chim. Acta* **2004**, *511*, 197-205.
19. Breznová, H.; Volf, R.; Král, V.; Sessler, J. L.; Try, A. C.; Shishkanova, T. V. Monomer and polymer quinoxaline derivatives for cationic recognition. *Anal. Bioanal. Chem.* **2003**, *375*, 1193-1198.
20. Koryta, J.; Štulík, K. *Iontově-selektivní elektrody*; Academia: Prague, 1984.
21. Tsagatakis, J. K.; Chaniotakis, N. A.; Jurkschat, K. 190. Multiorganyltin Compounds. Designing a novel phosphate-selective carrier. *Helv. Chim. Acta* **1994**, *77*, 2191-2196.
22. Chaniotakis, N. A.; Park, S. B.; Meyerhoff, M. E. Salicylate-Selective Membrane Electrode Based on Tin(IV) Tetraethylporphyrin. *Anal. Chem.* **1989**, *61*, 566-570.
23. Shahrokhian, S.; Hamzehloei, A.; Bagherzadeh, M. Chromium(III) Porphyrin as a Selective Ionophore in a Salicylate-Selective Membrane Electrode. *Anal. Chem.* **2002**, *74*, 3312-3320.
24. Lingenfelter, P. T. Electronic and ionic sensitivity of bilayers of poly(3,4-ethylenedioxythiophene) and poly(dibenzo-18-crown-6), Thesis, Laboratory of Analytical Chemistry, Abo Akademi University, Turku, Finland, 2000.
25. Mousavi, Z.; Bobacka, J.; Lewenstam, A.; Ivaska, A. Response mechanism of potentiometric Ag<sup>+</sup> sensor based on PEDOT doped with silver hexabromocarbonane. *J. Electroanal. Chem.* **2006**, *593*, 219-226.
26. Koziel, K.; Lapkowiski, M.; Lefrant, S. Spectroelectrochemical investigation of the memory effect in polyaniline. *Synthetic Metals* **1995**, *69*, 217-218.

27. Odin, C.; Nechtschein, M. Memory effect on conducting polymers; Electrochemical and ESP studies on polyaniline. *Synthetic Metals* **1991**, *41-43*, 2943-2946
28. Bobacka, J.; Ivaska, A.; Grzeszczuk, M. Electrochemical study of poly(3-octylthiophene) film electrodes. I. Electrolyte effects on the voltammetric characteristics of the polymer. *Synthetic Metals* **1991**, *44(1)*, 9-19.
29. Dong, S.; Sun, Z.; Lu, Z. A new kind of chemical sensor based on CP film. *J. Chem. Soc. , Chem. Commun.* **1988**, 993-995.
30. Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. Teaching old indicators new tricks. *Acc. Chem. Res.* **2001**, *34*, 963-972.
31. Esteves, V. I.; Lima, S. S. F.; Lima, D. L. D.; Duarte, A. C. Using capillary electrophoresis for the determination of organic acids in Port wine. *Anal. Chim. Acta* **2004**, *513*, 163-167.
32. Horie, H.; Yamauchi, Y.; Kohota, K. Analysis of organic anions in tea infusions using capillary electrophoresis. *J. Chromatogr. A* **1998**, *817*, 139-144.
33. Saavedra, L.; García, A.; Barbas, C. Development and validation of a capillary electrophoresis method for direct measurement of isocitric, citric, tartaric and malic acids in orange juice. *J. Chromatography A* **2000**, *881*, 395-401.