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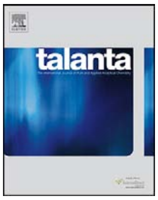

Discrimination of apple juice and herbal liqueur brands with solid-state electrodes covered with polyaniline and thiacalixarenes

G.A. Evtugyn[a,*], S.V. Belyakova[a], R.V. Shamagsumova[a], A.A. Saveliev[b], A.N. Ivanov[a], E.E. Stoikova[a], N.N. Dolgova[a], I.I. Stoikov[a], I.S. Antipin[a], H.C. Budnikov[a]

[a] A.M. Butlerov' Chemistry Institute, Kazan State University, 18 Kremlevskaya Street, Kazan 420008, Russian Federation
[b] Department of the Modeling of Ecological Systems of Kazan State University, 18 Kremlevskaya Street, Kazan 42008, Russian Federation

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ABSTRACT

Solid-contact ion-selective electrodes based on glassy carbon electrode covered with electropolymerized polyaniline and tetrasubstituted thiacalix[4]arene ionophores with hexyl and *o*-pyridylamido functional groups at the lower rim have been developed and examined in the discrimination of the brands of apple juices and herbal liqueurs. For this purpose, the liquids tested were diluted and spiked with a constant amount of Fe^{3+} ions. The variation of the signal toward Fe^{3+} ions was achieved due to their involvement in the reactions with the organic ligands and the antioxidants present. As was shown, the combination of the three electrodes with various receptors makes it possible to predict the brand of apple juices and herbal liqueurs using linear discriminant analysis in 95–100% cases. The discrimination procedure makes it possible to discriminate liquids within 20 min. Besides, the electrodes developed make it possible to detect individual antioxidants (ascorbic, malic, oxalic acids, hydroquinone, and quercetin) in the range from 5.0×10^{-6} to 1.0×10^{-2} M in direct potentiometric measurements and redox titration.

1. Introduction

Increasing demands for fast and inexpensive methods for the assessment of food quality stimulate intensive development of sensor devices applicable for this purpose [1]. The analysis of volatile substances in foodstuffs with gas sensors and multisensor systems, e.g. electronic noses, makes it possible to detect bacterial spoiling or classify aroma and other qualities of foodstuffs [2–4]. Meanwhile, the analysis of liquids becomes more and more important. In comparison with conventional analytical tools applied for this purpose, e.g. chromatography, IR and UV spectroscopy, sensors have milder demands to labor staff and offer extended opportunities for continuous monitoring as well as semi-quantitative testing.

From 1985 [5], various sensor systems have been developed for food analysis. At first, multisensor systems were based on voltammetric techniques [6–8]. Simultaneously, potentiometric sensor systems also called electronic tongue with conventional and/or specially designed ion-selective electrodes (ISEs) have been developed and successfully applied for food quality assessment [9–11]. Two of them, i.e. taste sensor based on planar electrodes covered with lipid membranes (Taste Sensing System SA 401, Anritsy Corp. [9]) and liquid and taste analyzer with a set of silicon transistors (α-Astree, Alfa MOS [12]) are commercially available. Photovoltaic cells [13] and SAW sensors [14] were also examined for the characterization of various complex samples. The signals of the sensors with cross-selectivity toward analyte components are commonly processed using various chemometric approaches for pattern recognition like principal component analysis (PCA), linear discriminant analysis (LDA), etc. [15,16]. The efficiency of discrimination of the foodstuffs was proved by comparison of the results obtained with multisensory systems to those of FTIR spectroscopy, HPLC [17] and sensory panel [18].

Solid-contact sensors based on polymeric coatings with implemented ionophores directly attached to the electrode surface provide some advantages over conventional membrane ISEs with internal filling and a reference electrode. They are easier to manufacture and operate and flexible in the geometry and dimension of a sensing surface. Thus, a microsensor array of solid-contact sensors was produced by printed circuit board technology on a solid support. Au microdisk transducers were covered with plastic membranes containing plasticizers, conventional ionophores toward some metal cations and inorganic anions and lipophilic salts. The sensor array were tested for the determination of ammonia and some alkali and alkali-earth metals and then used for the discrimination of juices [19] and milk brands [20]. Polyaniline (PANI) covered with a PVC membrane containing ionophore was used in all-solid ISEs for detecting K^+ [21], Ca^{2+} ions [22] and

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* Corresponding author.
E-mail address: Gennady.Evtugyn@ksu.ru (G.A. Evtugyn).

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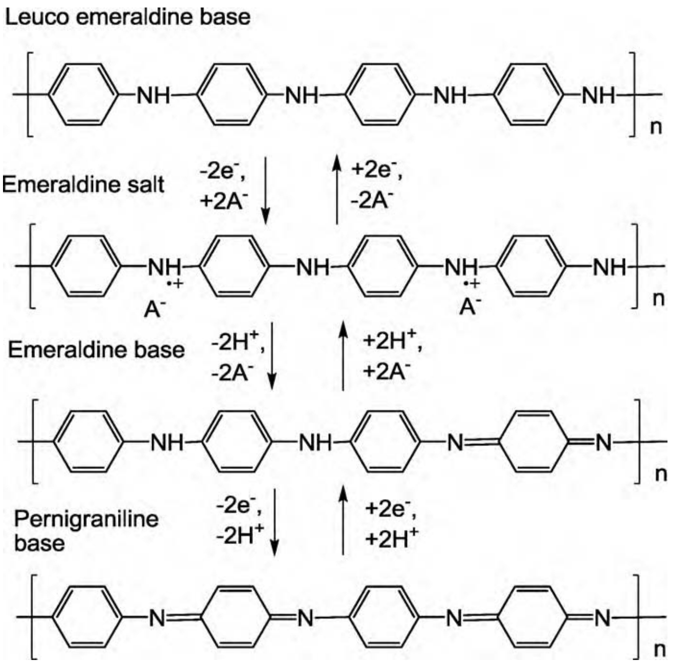
for pH measurement [23]. Glassy carbon electrode covered with electropolymerized PANI and tetrasubstituted thiacalix[4]arenes showed a potentiometric response to Ag^+ and Fe^{3+} ions [24]. Polypyrrole and polythiophene based solid-contact sensors were developed for determining the Ag^+ [25–28], Zn^{2+} [29], and K^+ ions [30]. Screen-printed carbon electrodes modified with PANI and polypyrrole were used for discrimination of the tea and coffee brands [31]. PANI and polypyrrole derivatives were investigated as chemiresistors for discriminating volatile organic compounds [4,32] and olive oil samples [32].

The idea to use electroconductive polymers (PANI, polypyrrole and polythiophene) in the assembly of the solid-state potentiometric sensors is based on the dependence of their redox equilibria on the charge transfer at the electrode surface. For PANI, the reactions assume the pH-dependent conversion of the electroconductive half-oxidized emeraldine salt into leucoemeraldine and pernigraniline (1) [23]. In conventional conditions of electropolymerization performed in the presence of strong mineral acids, emeraldine salt prevails over the other forms.



(1)

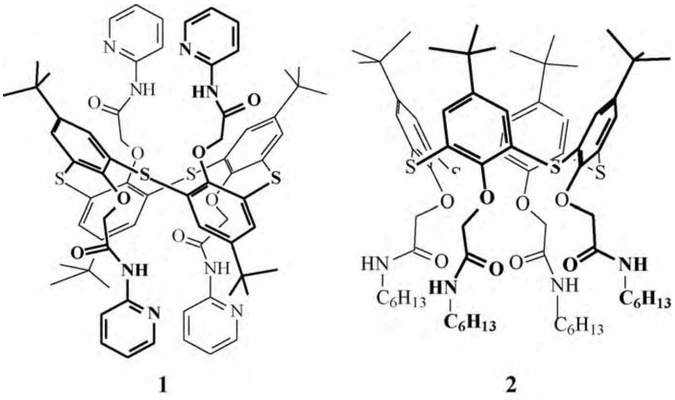
The reactions are complicated by the reversible acid–base transitions of appropriate forms as shown for emeraldine. For this reason the stationary potential of PANI depends on the pH and redox characteristics of the media. Thus, PANI doped with camphorsulfonic acid changed its transmembrane potential in the presence of some redox active compounds (ascorbic acid, Neutral red, Nile blue and N-phenylanthranilic acid) [33]. Stainless steel electrodes covered with PANI exhibited potentiometric response toward hydroquinone, $Fe^{2+/3+}$ and $[Fe(CN)_6]^{3-/4-}$ redox pairs. They were also used as indicator electrodes in the potentiometric titration of HCl with NaOH (neutralization reaction), and Fe^{2+} with Ce^{4+} or $KMnO_4$ (redox reaction) [34].

The pH- and redox-sensitivity of the PANI potential is commonly considered as a weak point of appropriate ISEs involved neutral ionophores for individual ions. Meanwhile it might prove an advantage for the samples containing antioxidants and metal complexing ligands. In this case, the response of the PANI based ISEs can be applied for the general assessment of the samples in accordance with their antioxidant activity or for their discrimination. In this work, we have shown the possibility of using PANI based ISEs with thiacalix[4]arene ionophores for the discrimination of the brands of fruit juices and herbal liqueurs.

2. Experimental

2.1. Reagents

The thiacalix[4]arenes **1** and **2** (2) were synthesized from the tetraacid derivative of *p*-*tert*-butylthiacalix[4]arene as described in [24,35]. PANI surface layer was obtained by electropolymerization from aniline freshly distilled under vacuum and stored under argon at 4 °C. Ascorbic acid was bought from Fluka. All the other reagents were of analytical grade.



(2)

2.2. Potentiometric sensor preparation and evaluation

The manufacture and performance of the ISE based on PANI and thiacalix[4]arene **1** were described previously [35]. The deposition of the ionophore **2** onto the PANI covered electrode was performed in a similar way. Prior to use, glassy carbon electrode of 2.7 mm in diameter was mechanically polished and cleaned with acetone, KOH and H_2SO_4. After that, the electrode was conditioned in 0.2 M H_2SO_4 at +0.1 V vs. Ag/AgCl for 3 min. PANI was polymerized during 10 min by multiple cycling of the potential between −0.3 and +0.8 V at 50 mV s^{-1} in 0.2 M H_2SO_4 containing 0.07 M aniline. Voltammetric experiments were conducted with AUTOLAB PGSTAT 302 (Ecochemie, the Netherlands). Pt wire was used as auxiliary electrode and Ag/AgCl as a reference electrode. After the PANI polymerization, 10 μL of 1.0×10^{-3} M solution of ionophore **1** or **2** in chloroform were placed onto the electrode surface and let dry at room temperature. The electropolymerization conditions as well as optimal surface concentration of thiacalix[4]arenes were established previously to reach maximal reproducibility and fast response [24,35]. When stored in dry conditions, the ISEs were conditioned in 0.1 M Na_2SO_4 for 20 min prior to use.

Three types of ISEs were used for the discrimination of juices and herbal liqueurs, i.e. one ISE covered with electropolymerized PANI only and two ISEs containing thiacalix[4]arenes **1** and **2** onto the PANI film. Between measurements, the ISEs were conditioned in 0.1 M Na_2SO_4 for 20 min. Changes in the stationary potential were measured for each ISE in three replications and used for antioxidant quantification and liquid discrimination without intermediate averaging.

All the potentiometric measurements were performed against the Ag/AgCl reference electrode in a non-thermostated 10 mL cell at room temperature. The unbiased potentiometric selectivity coefficients $\log K_{Fe/Me}$, were determined by the separate solution method

Table 1
Unbiased selectivity coefficients $\log K_{Fe/Me}$ of potentiometric sensor with thiacalix[4]arenes **1** and **2**.

Me	$\log K_{Fe/Me}$		Me	$\log K_{Fe/Me}$		Me	$\log K_{Fe/Me}$	
	1	**2**		**1**	**2**		**1**	**2**
K^+	−3.96	−11.22	Cu^{2+}	−3.55	−11.52	Ag^+	3.48	1.43
Li^+	−4.95	−11.10	Cd^{2+}	−6.66	−14.20	Pb^{2+}	−6.06	−13.20
Na^+	−2.91	−11.04	Zn^{2+}	−5.16	−12.78	Hg^{2+}[a]	2.3	3.35

[a] Measurements in 0.1 M HNO_3.

(SSM) with 0.01 M solutions of Fe^{3+} and interfering ions [36] in accordance with Eq. (3):

$$\log K_{Fe/Me} = \frac{zF(E^0_{Me} - E^0_{Fe})}{2.303RT} \tag{3}$$

where E^0_{Me} and E^0_{Fe} are the potential values measured for the interfering ion, Me^{z+}, and Fe^{3+}, respectively, and extrapolated to unit activity and z is the charge of the primary ion.

2.3. Juice and herbal liqueur testing

Two sets of samples were used for discrimination, i.e. commercial 200 mL packages of apple juice and 250–500 mL bottles of herbal liqueurs (alcohol content 28–45%) available on local market. For testing, samples were diluted with 1.0 mM Na_2SO_4. After that, $Fe(NO_3)_3$ was added to its concentration chosen to be constant in a series of measurements. All the reactions between the Fe^{3+} and sample components were performed within 10 min prior to the contact of the ISEs with spiked samples. This allowed avoiding probable complications related to the mixed character of the ISE potential and distortion of its reversibility.

No pH correction was made prior to measurements. The potentials of the ISEs were measured vs. the Ag/AgCl reference electrode under stirring. Also, pH and antioxidant capacity were measured for undiluted samples. The total antioxidant capacity was determined coulometrically with electrogenerated bromine in accordance with [37]. The amount of antioxidant was expressed by the charge required for generation of equivalent quantity of bromine, coulombs per 100 mL of undiluted sample.

The aliquots of juice were taken from packages just after their opening so that no effect of oxygen or light on the juice could be expected. Moreover, special series of measurements was performed to be sure that the uncertainty in the juice quality within the set of the packages did not affect the quality of discrimination. Thus, it was shown that the use of samples taken from various packages did not alter the signals of ISEs and discrimination quality in comparison with those performed with the samples from a single juice package.

2.4. Data processing

For statistical analysis, free software environment R 2.7.2 was used. Repetitive random resampling with replacement of the data set (bootstrapping [37]) was used for each sensor to reach close approximation to the true distribution. For principal component analysis (PCA) plots, the ClusPlot method of the Cluster package was used. The linear discriminant analysis (LDA) was performed using *lda* library of MASS package with 300 random sets obtained by bootstrapping and subdivided into training and testing sets. The statistical analysis was performed at a significance level of 5% in all the cases.

3. Results and discussion

3.1. ISE performance

The ISEs based on PANI and thiacalix[4]arenes **1** and **2** showed selective response toward Ag^+, Hg^{2+} and Fe^{3+} ions over the other alkali and most transient metal ions tested. The unbiased selectivity coefficients $\log K_{Fe/Me}$ calculated by SSM are presented in Table 1. Measurements with mercury salt were performed in 0.1 M HNO_3 because of the hydrolytic instability of Hg^{2+} ion in neutral media. Both receptors showed high selectivity toward the ions investigated except Ag^+ and Hg^{2+}. The sensitivity of the ISE potential to Ag^+ and Hg^{2+} is mainly related to the multipoint interactions of the ions with the heteroatoms of amide groups of the substituents at the lower rim and the sulfur bridging atoms of the macrocycle. Also, geometric conformance of the ion radius and the size of the binding site of thiacalixarene moiety are important (see expected structure of host–guest complex in [24]). Contrary to that, alkali metals are primarily coordinated by carbonyl oxygen atoms. This provides the predominant binding of soft transient metals (Hg^{2+} and Ag^+ ions) over alkali metals.

The potentiometric selectivity coefficients provide an estimate of thiacalixarene affinity toward various metals because the partial hydrolysis of Fe^{3+} followed by the formation of hydroxy ions, e.g. $Fe(OH)^{2+}$, are not taken into account. The higher selectivity of thiacalixarene **2** against thiacalixarene **1** can be related to the shielding effect of lipophilic alkyl substituents. A more extended discussion of the factors affecting the potentiometric selectivity of calixarene based solid-contact sensors and the selectivity coefficients calculated for Ag^+ primary ion more stable in the measurement conditions are given in [24] for thiacalix[4]arenes with morpholide, pyrrolidide, amidopyridide and hydrazide functional groups at the lower rim in *cone*, *partial cone* and *1,3-alternate* conformations.

As regards the Fe^{3+} ion, its effect on the ISE potential is partially established by its involvement in PANI redox equilibria altered by host–guest complexation. The appropriate calibration curves were S-shaped with the maximum shift of the potential of about 400 mV which corresponded to the difference in standard redox potentials of Fe(III)/Fe(II) and that of PANI. Thiacalix[4]arenes **1** and **2** alter the access of Fe^{3+} ions to the PANI and its reactivity in appropriate redox reactions.

The performance of solid-state potentiometric ISEs depends on the reversibility and flexibility of PANI redox and acid–base equilibria. Prior to thiacalixarene deposition, PANI showed a Nernstian slope of pH dependence of the potential (52–56 mV/pH in the pH range 2.0–7.0) and a good recovery of the response in the series of alternating pH changes (R.S.D. 1.5% for six consecutive measurements of the potential at pH 3.0 and 6.0 with the same ISE). The interaction of PANI with thiacalixarenes **1** and **2** decreased the pH sensitivity of the signal to 35 and 45 mV/pH, respectively. Redox reversibility was estimated in a similar manner by measuring the potential of the ISE in the mixture of $[Fe(CN)_6]^{3-/4-}$ of the total concentration of 1.0×10^{-2} M and the different ratio of reduced and oxidized form (from 5:1 to 1:5). The slope of the appropriate

Table 2
Analytical characteristics of antioxidant determination with solid-contact ISEs based on PANI and thiacalix[4]arene receptors in the presence of Fe^{3+} ions.

Antioxidant	Surface layer	Linear range, mM	Slope, mV per decade	Limit of detection, mM
Ascorbic acid	PANI	0.1–1	28 ± 2	0.05
	PANI + **1**	0.05–1	35 ± 2	0.02
	PANI + **2**	0.04–2	34 ± 2	0.02
Malic acid	PANI	0.5–10	47 ± 3	0.1
	PANI + **1**	0.1–10	35 ± 3	0.05
	PANI + **2**	0.1–10	40 ± 3	0.05
Oxalic acid	PANI	0.5–10	46 ± 3	0.1
	PANI + **1**	0.05–5	61 ± 3	0.02
	PANI + **2**	0.04–4	80 ± 4	0.02
Hydroquinone	PANI	0.1–4	25 ± 2	0.05
	PANI + **1**	0.03–2	33 ± 2	0.01
	PANI + **2**	0.02–1	34 ± 2	0.01
Quercetin	PANI	0.01–2	34 ± 2	0.005
	PANI + **1**	0.01–2	47 ± 3	0.005
	PANI + **2**	0.005–1	45 ± 3	0.002

dependence of the potential was equal to 50 mV for PANI and 45 mV for PANI–**1** and PANI–**2** coatings.

3.2. Signal reproducibility

All the signals of the ISEs toward metal ions were reversible. After measurement, the ISEs were recovered by washing in 0.1 M Na_2SO_4 for 10 min. The procedure can be accelerated by the addition of EDTA or hydroquinone but this is not mandatory. In 10 consecutive measurements of alternating Fe^{3+} concentrations (1.0×10^{-4}–1.0×10^{-3} M) in model aqueous solution, the deviation of the signals did not exceed 3.5% for 0.1 mM Fe^{3+} and 2.4% for 1.0 mM Fe^{3+}. For a set of six different ISEs with the same ionophore, the R.S.D. was 5.6% for 1.0×10^{-3} M Fe^{3+}.

The reproducibility of the ISE signal in apple juices diluted in ratio from 1:1 to 1:100 with 1.0 mM Na_2SO_4 was estimated in the presence of a constant concentration of Fe^{3+}. Thus, for 1:20 dilution the R.S.D. of the signal toward 1.0 mM Fe^{3+} was about 4–5% (six replications). The lower the dilution the higher was the deviation of the signal up to the maximum of 6–8% for 1:1 dilution. This could be due to the effect of heterogeneity of the sample and the sorption of Fe^{3+} onto the fruit tissue particles. To avoid the uncertainty of the signal measured, the minimal dilution of 1:20 was chosen for further experiments. For diluted liqueurs, the R.S.D. of the signal did not differ significantly from that observed in 1.0×10^{-3} M Na_2SO_4.

No significant drift of the background potential was observed within a working day for about 20 consecutive measurements with the same electrode in the 1.0×10^{-4}–1.0×10^{-3} M Fe^{3+} solution. For Ag^+ ions, the potential of the electrode increased by 1–2% after each recovery. Nevertheless, the shift of the potential after the Ag^+ injection remained quite stable for at least 10 measurements with the same electrode.

All the ISEs developed retain their response toward Fe^{3+} ions, pH and redox potential during at least three moths of storage either in dry conditions or in 1.0×10^{-3} M Na_2SO_4. A drift of stationary potential of about 1–1.5 mV per day can be observed in the latter case which does not affect the response toward 1.0×10^{-3} M Fe^{3+}. When stored for more than one day in dry conditions, the ISEs should be conditioned in 1.0×10^{-3} M Na_2SO_4 containing 1.0×10^{-2} M Fe^{3+} prior to use. No significant effect of storage period on potentiometric selectivity of the response was observed.

3.3. Antioxidant determination

The involvement of Fe^{3+} ions in the redox conversion of PANI makes it possible to use the PANI based ISEs for detecting antioxidants. For this purpose, the ISE was first equilibrated in Fe^{3+} solution and then an antioxidant was added and a sharp decay of the potential was recorded. The response toward ascorbic acid, hydroquinone, quercetin, malic and oxalic acids was found to be rather fast enough (within 2 min) and reversible. The analytical characteristics of antioxidant determination are summarized in Table 2. The limit of detection (LOD) was determined using S/N = 3.0 ratio. The slope of appropriate calibration curves varied from 25 to 28 mV, which nearly corresponded to the transfer of two electrons, and 60 mV per decade assuming a one-electron oxidation. The super-Nernstian slope observed in some cases can be probably referred to the mixed complexation of Fe^{3+} ions with organic acids and a thiacalixarene receptor which promotes redox reactions onto the PANI surface. A similar behavior of Fe^{3+}–thiacalixarene–picrate triple complexes is used in the investigation of complexation by the picrate extraction method [24]. The thiacalixarenes improve the sensitivity of antioxidant determination in comparison with the PANI covered electrodes. The analytical characteristics of individual compounds are comparable with those obtained by other solid-contact potentiometric sensors [34,38]. Thus, the ISEs based on screen-printed carbon electrodes covered with PVC membrane containing Ph_3SnCl as an ascorbate ionophore gave linear range of concentrations from 0.02 to 3 mM and response time of 5–10 min [38]. It should be mentioned that ascorbic acid, hydroquinone and quercetin diminish the potential of PANI in the absence of Fe^{3+} ions, as well. However, the response is less reproducible (R.S.D. 7–9%) and less sensitive (slope about 22–25 mV per decade) than that obtained in the presence of Fe^{3+} ions. The other organic acids tested as well as glucose did not alter the PANI potential up to their concentration of 10 mM.

The ISE with thiacalixarene **1** was examined as an indicator electrode in the potentiometric titration of ascorbic acid. A remarkable shift of the potential of about 100 mV was observed at the end point of titration. The tablets of ascorbic acid with label content of 250 mg of active substance (“Marbioprom”, Russia) were ground, dissolved in 0.2 M H_2SO_4 and titrated with a 10 mM $Fe(SO_4)_3$ solution. The average result was calculated from six replications. The content obtained (220 ± 14 mg) with the ISE coincided with that of iodometric titration [39] (235 ± 5 mg).

Certainly, the response of the ISEs toward antioxidants is non-selective. However, the dependence of the slope of the calibration curve on the thiacalixarene receptor and antioxidant suppose the influence of complexation stages on the redox processes and the resulting shift of the potential. This offers opportunities for the discrimination of the signals in the liquids containing various active ingredients affecting the signals of the ISEs developed.

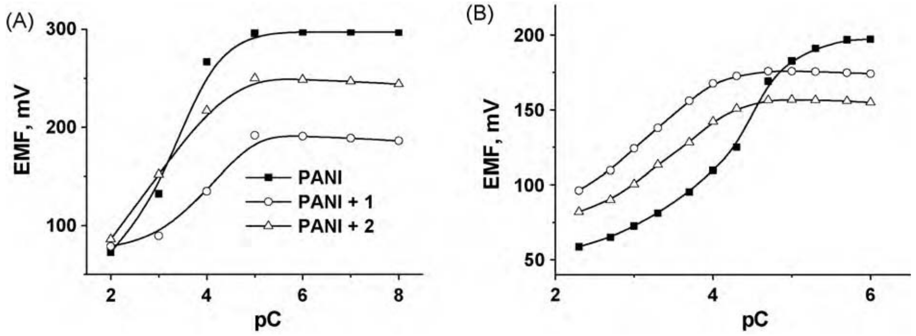


Fig. 1. The dependence of the ISE signals on the concentration of ascorbic acid in spiked apple juices "Rich" (A) and "Orchard" (B) diluted in 1:20 (v:v) ratio with 1.0×10^{-3} M Na_2SO_4. Measurements in the presence of 1.0×10^{-4} M $Fe(NO_3)_3$.

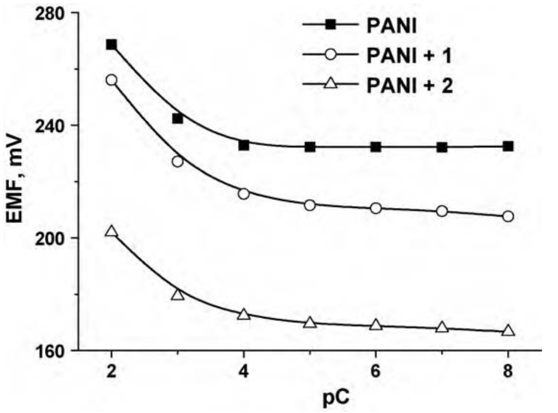


Fig. 2. The effect of sodium citrate on the signal of Fe^{3+} ions in spiked apple juice "Rich" diluted in 1:20 (v:v) ratio with 1.0×10^{-3} M Na_2SO_4. Measurements in the presence of 1.0×10^{-4} M $Fe(NO_3)_3$.

3.4. Spiked juices

After testing individual antioxidants, the ISEs developed were used for the determination of active ingredients in spiked juices. Commercial 200 mL packages of fruit juices were spiked with additional amounts of antioxidants and organic acids exceeded their natural content. The calibration curves of antioxidants depended both on the juice brand and the thiacalixarene receptor (e.g. see Fig. 1 for apple juices "Rich" and "Orchard" spiked with various amounts of ascorbic acid). To avoid the effect of juice heterogeneity and improve repeatability of the signal, samples were diluted with 1.0 mM Na_2SO_4. The concentration of the Fe^{3+} ions added was specified to obtain maximal difference in the ISE responses. A similar behavior was observed for other antioxidants, i.e. hydroquinone and quercetin (not shown).

Sodium citrate gave unexpected result. The signal increased with citrate concentration as shown in Fig. 2 for the "Rich" apple juice. Citric acid contains three carboxylic groups and can form several negatively charged complexes with Fe^{3+} ions. Their charge and stoichiometry depend on the metal concentration and pH ($[Fe(cit)_2]^{5-}$, $Fe_2(cit)_2{}^{2-}$, etc. with (cit) as a citrate ligand [40]). The complexes retain the ability of redox reactions. The formation of citrate complexes could result in their electrostatic accumulation onto the positively charged PANI layer.

3.5. Discrimination of apple juice brands

The discrimination of apple juices was performed with six brands available on local market ("Orchard", "Rich", "Tropicana", "My Family", "Tonus", and "Good"). No significant difference in the signals measured in 10 various 200 mL packages of the juices was found for all the ISEs and juice brands (R.S.D. of about 3.5%). Before testing, the juice was multiply diluted with 1.0 mM Na_2SO_4 and a constant amount of $Fe(NO_3)_3$ was added to each diluted sample. No additional treatment such as pH adjustment or sample filtering was required. The results of the measurements performed with different Fe^{3+} concentrations are presented for two juices, "Orchard" and "Rich", in Fig. 3. Both the absolute values of the ISE signals and the shape of curves recorded with various ISEs depended on the juice brand and dilution. The comparison of the antioxidant capacity of juices obtained by coulometry with electrogenerated bromine with the signal characteristics did not reveal any correlation. This reflects the complex influence of juice components on the signal toward Fe^{3+} ions which can involve both the complexation of the ion, its redox conversion and alteration of accumulation in the reaction with thiacalix[4]arene ionophores.

The discrimination of the juices in accordance with their brands was investigated using PCA. For this purpose, ISE signals were measured for two Fe^{3+} concentrations (1.0×10^{-3} and 1.0×10^{-4} M) and

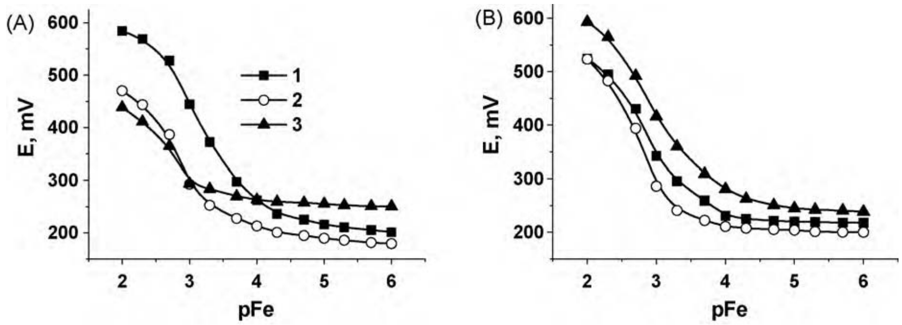


Fig. 3. The dependence of the signal of ISEs based on PANI (1), PANI–thiacalixarene **1** (2) and PANI–thiacalixarene **2** (3) on the Fe^{3+} concentration. Apple juice brands: (A) "Orchard"; (B) "Rich". Juices were diluted in the ratio 1:20 with 1.0×10^{-3} M Na_2SO_4.

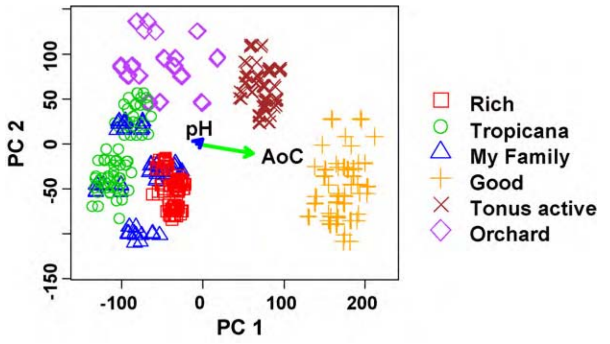


Fig. 4. PCA scores plot (two first principal components) of apple juice brands, sample dilution 1:70 and 1:100, Fe^{3+} concentration 1.0×10^{-3} M.

for several juice dilutions. All the measurements were performed in three replications made with individual ISEs (nine sensors in total, i.e. three with PANI layer, three with PANI and ionophore **1** and three with PANI and ionophore **2**). Thus, each variable set comprised $n \times m \times 9$ values where $n = 1$ or 2 is the number of dilutions and $m = 1$ or 2 is the number of Fe^{3+} concentrations. The comparison of the results obtained with different combinations of parameters showed better discrimination for the sets obtained with 1.0×10^{-3} M Fe^{3+} and a high enough juice dilution (1:70 and 1:100), e.g. see Fig. 4. This probably corresponds to the extended involvement of the juice components in the reaction with Fe^{3+} in accordance with the stoichiometry of the reactions and natural content of antioxidants and organic acids in the juices tested. The variance explained by these two components was 78.8%.

“My Family”, a freshener with 25% of natural apple juice, was the only one brand which could not be specified by PCA. This might be due to lesser stability of the samples and their higher heterogeneity caused by additives in comparison with other samples tested. For other brands of apple juice, the explained variance by two PCA scores increases to 95.2%. The arrows on the plot represent the trends of total antioxidant capacity (AoC) and pH measured in undiluted juices. Thus, “Tonus active” and “Good” can differ from other brands due to increased amounts of additives (predominantly minerals and vitamins) affecting their antioxidant capacity. For other juices, especially “Rich” and “Tropicana” which are packaged with no preservatives, the differentiation can be mainly referred to Fe^{3+} complexation. Contrary to that, pH value does not affect the discrimination, probably due to narrow range of changes within the set of samples tested. An increase of the number of variances improves the discrimination of the juices. However, this complicates measurements and is time consuming. Thus, an increase in the variable set up to 36 measurements (two dilutions and two Fe^{3+} concentrations) improves the variance explained by two principal components to 97.5%. However, this increases twice the time necessary for discrimination and complicates measurement protocol in comparison with conventional electronic tongue systems [15–20].

The possibility for discrimination of apple juice brands was confirmed by LDA. In this method, learning and testing sets were randomly generated using bootstrapping [41] and the frequency of correct prediction of a juice brand was calculated. The average proportion of true prediction varied from 73 to 100% for various initial data sets. Better results corresponded to data sets which showed maximum explained variance for two PCA components for the set. Also, the influence of the data set size on the prediction quality was considered. The use of single sample dilution and Fe^{3+} concentration (nine measurements) provided the proportion of the true prediction of the juice brand from 73 to 93%. The extension of the initial data set by the results obtained with the second concentration of Fe^{3+} ions (18 measurements in total) improved the performance to 97–100%.

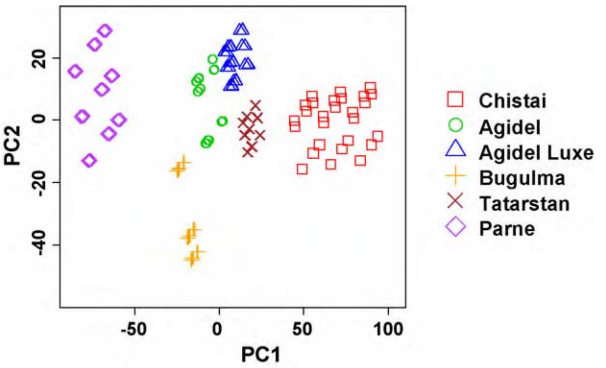


Fig. 5. PCA scores plot (two first principal components) of herbal liqueurs, sample dilutions 1:10 and 1:20, Fe^{3+} concentration 1.0×10^{-4} M.

3.6. Discrimination of herbal liqueurs

All the liqueurs tested were produced in the local area of Russian Federation (Volga region, Tatarstan and Bashkortostan Republics) from alcoholic extracts of medicinal herbs and roots with addition of natural flavorings. The liqueurs contained 28–40% of ethanol and 10–20% of sugar. In preliminary experiments, no effect on the ISEs signal toward Fe^{3+} ions was found for similar quantities of ethanol and glucose in model aqueous solutions. Multiple signal measurements in undiluted samples of liqueurs and 40% aqueous ethanol reduced the lifetime of the ISEs and caused a regular drift of their stationary potential to lower values due to partial degradation of the surface layer. Changes in the Fe^{3+} calibration curves obtained with various ISEs in diluted liqueurs took place in a narrow enough interval of dilutions (1:1–1:20 vs. 1:20–1:100 for apple juices). Probably this is due to the lower antioxidant capacity of the liqueurs in comparison with the apple juices tested. Best results were obtained with 1:10–1:20 dilution (see an example of PCA scores plot in Fig. 5). The efficiency of liqueur brand prediction with LDA exceeded 82% in case of 9 measurements and 92% for 18 measurements (two dilutions of liqueurs).

4. Conclusion

ISEs based on PANI and thiacalixarenes **1** and **2** showed a remarkable response toward Fe^{3+} ions related to the reversible redox conversion of PANI which can be affected by complexation of the indicator ion with thiacalixarenes and some organic ligands present in the samples tested. The variety of such factors affecting the response of ISEs made it possible to suggest the combination of three ISEs for discrimination of some foodstuffs rich in antioxidants and organic acids. The application of low-selective sensors in food analysis has been described from 1990s (electronic tongue concept, see recent review [44] and references therein). The novelty of the approach suggested involves the measurement protocol requiring the addition of a redox indicator, Fe^{3+} ion, in combination with PANI sensitive to redox potential of the media.

The following reactions can be responsible for altering the Fe^{3+} signal and discrimination of juices and liqueurs described in this work:

- partial oxidation of readily oxidizable components by the Fe^{3+} ions;
- changes in pH and redox potential due to hydrolytic instability of the Fe^{3+} ions affected by organic ligands present in the samples tested;

- complexation of Fe^{3+} ions with organic species such as natural organic acids, artificial food additives, antioxidants, and vitamins.

All of these reactions result in the changes of redox potential recorded with the PANI sensor. Even though the number of factors affecting the signal of ISEs developed seems rather high, the experiments showed predominant influence of readily oxidizable compounds and, to a less extent, of organic ligands. Contrary to that, pH of the juices and liqueurs varied in rather narrow range of values and did not alter the discrimination quality. It should be also mentioned that the discrimination results were found to be quite stable if the samples were taken and tested within a working day. Probably, the influence of pH, storage time and measurement period would be of greater importance in other cases, e.g. in discrimination of freshly pressed juices or in testing packages stored in unsatisfactory conditions.

The ISEs developed can be also used for the determination of individual antioxidants in pharmaceutical preparations or as indicator electrodes in redox titration. The use of ISEs combination for the measurement of the redox potential of diluted juices and liqueurs spiked with a constant amount of Fe^{3+} ions provides their discrimination by a brand proved by PCA and LDA. Even though thiacalixarenes **1** and **2** were rather similar in their reactions toward Fe^{3+} ions, their signals in spiked samples did not correlate with each other. Probably, this could be due to the non-linear calibration plot of Fe^{3+} ions. In addition, long hydrophobic substituents of thiacalixarene **2** make it more sensitive toward lipophilic components of the samples.

The discrimination protocol seems more complicated than that suggested for fruit juices with traditional electronic tongue [16–18,42,43] because it involves additional sample treatment. However, this is compensated for by a much lower number of ISEs required (only three vs. more than ten in the e-tongues described). Taking into account the fast response (about 10–15 s), the total time required for testing one samples does not exceed 20 min. It should also be mention that the satisfactory discrimination of juice and liqueur brands is reached with a small enough number of replications. The low cost and simple procedure of the ISE manufacture offer opportunities for the development of single-use sensors based, e.g. on screen-printed electrodes. Other prospects of the system described call for extension of the features of artificial receptors used as ionophores.

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