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Potentiometric Electronic Tongue for Pharmaceutical Analytics: Determination of Ascorbic Acid Based on Electropolymerized Films

Gabriela Broncová *, Vadim Prokopec and Tatiana V. Shishkanova

Department of Analytical Chemistry, University of Chemistry and Technology, Technická 5, 166 28 Prague, Czech Republic; Vadim.Prokopec@vscht.cz (V.P.); Tatiana.Shishkanova@vscht.cz (T.V.S.)
* Correspondence: Gabriela.Broncova@vscht.cz; Tel.: +420-220-444-227; Fax: +420-220-444-058

Abstract: This work deals with the design of an experimental potentiometric electronic tongue (ET) for the recognition of various samples of effervescent tablets with different ascorbic acid (vitamin C) contents. The ET consisted of twelve potentiometric sensors based on conductive polymers, which were derived from 4-amino-2,1,3-benzothiadiazole, 3,4-diaminobenzoic acid, and neutral red on the surface of the platinum electrode using cyclic voltammetry. The aim of the potentiometric study was to assess the influence of the vitamin C content and the composition of the matrix of commercial samples on the potentiometric response. The results obtained from the sensor array proved that the stability of the potentiometric signal and the accuracy of measurements are affected by individual sensors. The identification of the vitamin C content in the individual samples of effervescent tablets obtained by means of the potentiometric electronic tongue corresponded with the results of the coulometric titration.

Keywords: electronic tongue; conducting polymer; electropolymerized film; ascorbic acid; effervescent tablet; stability

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1. Introduction

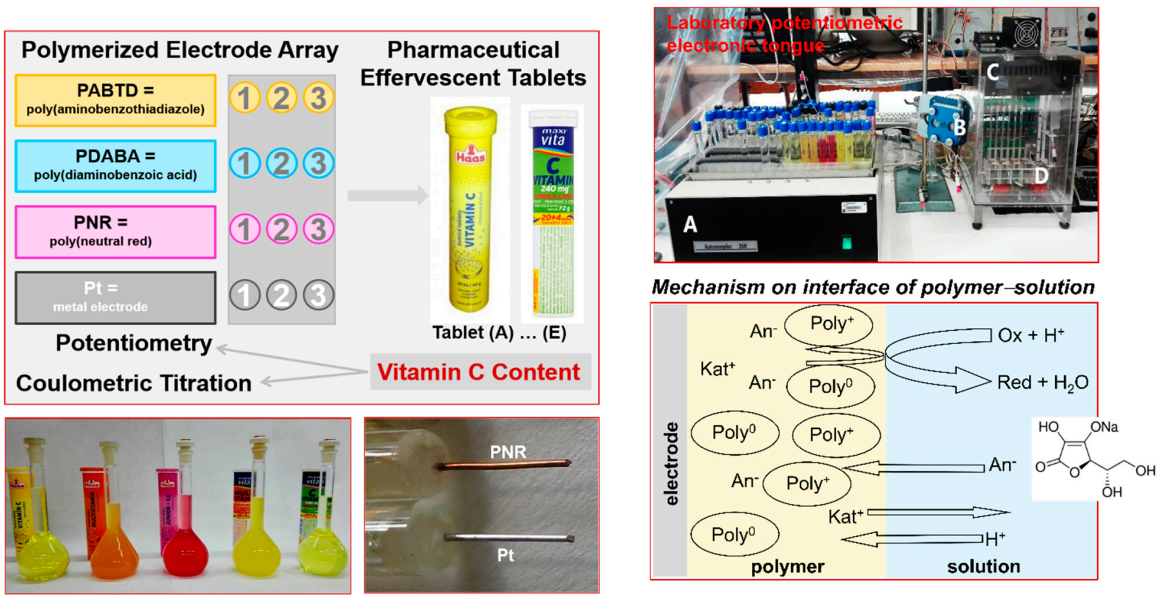
The sensor array (electronic tongue (ET)) allows different flavors of liquid samples to be distinguished. The taste is determined by the presence and amount of specific substances that can be detected most often electrochemically (potentiometrically, voltammetrically, and amperometrically) using membrane, metal, or polymeric electrodes [1,2]. Commercial ETs use potentiometric sensors based on lipids imitating biological membranes [3].

Taste is one of the basic characteristics that must be taken into account and characterized in the development of any drug. A large proportion of the active ingredients exhibit an unpleasant taste, and therefore it is necessary to mask it. One way of masking taste and inhibiting the taste receptor interaction with the active ingredient is to produce coated tablets or to complex them [4]. Some other masking strategies are based on the delusion of the taste sense, for example, by using sweeteners. The developed taste masking technologies have been described in detail by Sohi et al. [5], Ayenew et al. [6], Wagh and Ghadlinge [7], and Shishkanova et al. [8]. Ascorbic acid (AA) is one of the substances affecting the taste; it belongs to the therapeutic group of medicaments—vitamins—and can be recognized using ET [9]. Electronic tongue can be used for reliable detection of the taste and quality of the drug with a favorable ratio between the time-consuming requirements for the experiments and the costs incurred. Ascorbic acid is one of the first vitamins discovered. Its content in fruits and vegetables gradually decreases during their storage, and a more concentrated synthetic form needs to be used to supply the necessary amount of vitamin C to the body [10]. The properties of AA and its methods of determination are summarized in a review from 2018 [11] which describes in particular electrochemical techniques such as voltammetry and amperometry, as well as other ones (titration, chromatography, pho-

tometry, fluorimetry, and others). However, these methods are relatively costly, because they require the use of expensive equipment and reagents. Additionally, potentiometry is a very simple and cheap method including only a few types of electrode materials, e.g., an iodine-modified platinum electrode, graphite, and glassy-carbon electrodes coated with Prussian blue [12], graphite-epoxy composite electrode [13], and MnO_2 modified nanoparticles ISFET [14], which were studied as electrodes that gave potentiometric responses to AA. Electrodes containing these mediators can act as redox electrodes with respect to AA.

Conductive polymers (CPs) are very sensitive sensor materials that could be used for AA detection. For example, electropolymerized films from functionalized thiadiazole [15], aniline [16], and indicator [17] and other derivatives [18] were used for the simultaneous voltammetric determination of AA next to dopamine. Currently, according to the literature, CPs based on 3,4-ethylenedioxythiophene, pyrrole, aniline [19], porphyrins [20], and other non-traditional materials [21,22], may be of interest for the taste discrimination in the pharmaceutical industry. Flavor differentiation in pharmacy is aimed at optimizing the taste of medicaments. Development of effective methods and sensors for the determination of vitamin C is necessary in view of the continued importance and presence of this key analyte in food, medicaments, and biological fluids, with effects on redox processes, human health, and food quality [11].

The mechanism of AA detection by CPs deposited on Pt substrate can be explained based on the facts described by Maksimuk [23] and Bobacka [24] according to Figure 1b, as will be described below. All considerations of mechanisms can be reduced to two fundamental options of potential response. First, the potential response is driven by the change in potential at the conductive polymer–solution interface and is conditioned by the transfer of electrons and doping ions without changing the redox state of the polymer. Second, the potential response is the result of a redox process that changes the redox composition of the polymer surface due to the equilibrium between the polymer and the solution. Electrons are exchanged between the electrode and the polymer and between the polymer and the solution. The polymer undergoes redox reactions depending on the redox components present in the solution. If the polymer is in the oxidized state, then its charge is compensated by anions from the solution, which can be easily exchanged [23,24].



(**a**) (**b**)

Figure 1. Potentiometric electronic tongue (**a**) concept for discrimination of sample of effervescent tablets containing vitamin C; (**b**) laboratory ET setup (automatic sample feeder (A) connected with a pump (B) and a thermostatically controlled box (C), in which the cell (D) with electrodes is located) and schema of possible mechanism of electrode response [23,24].

The aim of this work was to develop sensor array based on Pt electrodes modified with 4-amino-2,1,3-benzothiadiazole [25], 3,4-diaminobenzoic acid [26], and neutral red [27] using the method of cyclic voltammetry. The potentiometric properties of the designed electrode array were studied and applied for testing and for relatively fast and precise discrimination of samples of effervescent tablets containing vitamin C (Figure 1) dissolved in different environments. The coulometric titration method was used as a comparative method.

2. Materials and Methods

2.1. Materials

Fluka chemicals, namely tetrabutylammonium perchlorate (Bu_4NClO_4) with a purity of ≥99% and acetonitrile with a purity of ≥99.5%, from Lachema (Brno, Czech Republic), neutral red (3-amino-7-dimethylamino-2-methylphenazine, abbreviated NR), and sulfuric acid with a purity of 96% and a density of 1830 g L^{-1}, 4% pure 4-amino-2,1,3-benzothiadiazole (abbreviated as ABTD), and 97% pure 3,4-diaminobenzoic acid (abbreviated as DABA) were used from Sigma-Aldrich (St. Louis, MO, USA). The nitrogen gas (N_2) bomb was supplied by Linde. Acids and various inorganic salts used in the potentiometric and coulometric measurements were of analytical grade from Lachema (Brno, Czech Republic). Five commercial effervescent tablets labelled as Haas (A, B, C) and MaxiVita (D and E) were purchased from local stores (Prague, Czech Republic). All working solutions were prepared using redistilled water.

2.2. Preparation of Polymerized Film Electrodes for Electronic Tongue

The experiment was performed using a potentiostat/galvanostat Autolab pGST-12 (Eco Chemie, Utrecht, The Netherlands). Cyclic voltammetry took place in a voltammetric cell with a working Pt wire electrode sealed in a glass tube, an Ag/AgCl electrode (3M KCl), and an auxiliary large-area Pt electrode. The polymerization conditions are summarized in the Table 1. Data acquired during the individual measurements were automatically saved in the Nova 1.10 program.

Table 1. Conditions for the preparation of three types of polymer films on Pt wire electrodes (n = 3).

Composition of the Polymerization Bath			Polymerization Parameters		
Monomer (Electrode with Polymer)	**Concentration of Monomer [mol L^{-1}]**	**Supporting Electrolyte**	**Potential Range vs. Ag/AgCl [V]**	**Number of Cycles**	**Scan Rate [mV s^{-1}]**
ABTD (PABTD_1, _2, _3)	0.005	3 M H_2SO_4	0.00 ÷ 1.25	25	50
DABA (PDABA_1, _2, _3)	0.005	0.5 M H_2SO_4	0.00 ÷ 1.20	20	50
NR (PNR_1, _2, _3)	0.005	acetonitrile, 0.05 M Bu_4NClO_4	−0.20 ÷ 1.80	20	50

2.3. Potentiometric Measurements with Electrode Array

An ion selective electrode (ISE) tester [28] was used for the potentiometric measurements. The tester consisted of an analog unit box, autosampler with sampling needle, hot-air thermostat, measuring cell, peristaltic pump, and a computer with a PC 516 data acquisition card. The electrode array was composed of twelve individual electrodes (three bare Pt electrodes and three electrodes for each type of polymer marked as: Pt_1, Pt_2, Pt_3, PDABA_1, PDABA_2, PDABA_3, PNR_1, PNR_2, PNR_3, PABTD_1, PABTD_2, and PABTD_3). The response of the electrodes was measured against the 3M Ag/AgCl electrode. The measurement of one tube took 454 s. The pH dependence was measured in 0.04 M Britton–Robinson buffer in pH range 2–11. Different pH values were obtained by adding 0.1 M NaOH solution from a burette; a pH meter was used for control. A series of ascorbic acid calibration solutions in pH 5 acetate buffer was prepared. The concentration

range was 10^{-6}–10^{-1} M. The pH value of each calibration solution was adjusted to 5 by adding a 0.1 M NaOH solution. The pH-adjusted calibration solutions were filled into tubes, which were further analyzed potentiometrically. For the measurement, it was also necessary to prepare solutions for washing the apparatus and the surface of the electrodes; tubes were prepared with redistilled water and acetate buffer pH 4.0. As part of the electrode stability measurements, the potential value for each electrode in the array in the acetate buffer with pH 4 was monitored for several days.

2.4. Analysis of Commercial Samples of Effervescent Tablets Containing Vitamin C Dissolved in Acetate Buffer or Distilled Water

Samples of effervescent tablets containing vitamin C were prepared by dissolving in either acetate buffer pH 5 or distilled water. The weighed tablets A, B, C, D, E were dissolved in an appropriate amount of about 30 mL of acetate buffer pH 5.0, or distilled water in a beaker, then the solution was quantitatively transferred to a 100 mL volumetric flask. The volume was made up to the mark with the same buffer/water. The pH was measured for each of the effervescent tablet solutions; the pH of the samples dissolved in redistilled water was no longer adjusted. The properties of the effervescent tablets in the acetate buffer solution and distilled water are summarized in Table 2.

Table 2. Weights and measured pH values of prepared solutions; description of the appearance of used effervescent tablets A–E (marked S_A–S_E) dissolved in acetate buffer or distilled water (n = 3).

Tablets	Weight [g]	Acetate Buffer pH	Redistill Water pH	Appearance of the Solution [1]
S_A	4.0147 ± 0.0124	4.98	4.06	Clear yellow
S_B	3.9736 ± 0.0112	4.97	4.05	Clear orange
S_C	3.9501 ± 0.0099	4.97	3.9	Cloudy pink, traces of filler
S_D	3.1566 ± 0.0119	4.98	3.77	Cloudy yellow
S_E	3.0553 ± 0.0095	5.01	4.32	Clear yellow

[1] Appearance of the sample solution (see Figure 1a below).

Potentiometric measurements of the selected samples of effervescent tablets dissolved in acetate buffer with electrode array were performed in the sequence listed in Table 3.

Table 3. Sequence samples of effervescent tablets for potentiometric measurements with polymerized electrode array, samples A–E are labelled S_A–S_E. The sequence was repeated 6 times (n = 6).

Position order	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Sample	0 [1]	AcB [2]	AcB	S_A	0	AcB	AcB	S_B	0	AcB	AcB	S_C	0
Position order	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**14**	**15**
Sample	AcB	AcB	S_D	0	AcB	AcB	S_E	AcB	AcB	0	0	AcB	AcB

[1] 0 is distilled water, [2] AcB is acetate buffer.

Samples of effervescent tablets dissolved in distilled water were processed in the same way. The samples were arranged in the same sequence according to Table 3.

2.5. Data Processing

Statistical processing of the measured potentiometric data was performed in The Unscrambler X 10.3 (CAMO AS, Oslo, Norway), a commercial software product for multivariate data analysis. All data sets were subjected to the exploratory PCA (Principal Component Analysis), using NIPALS (Nonlinear Iterative Partial Least Squares) algorithm with full cross-validation procedure for computation of principal component scores and loadings. The use of at least 3 principal components and the explanation of at least 95% input variability were determined as basic parameters for data set processing. In this work, the row and column in the matrix arrangement of multidimensional data refer to

the response of individual polymerized electrode depending on the type of the sample determined and to the type of polymer including into sensor array, respectively. Each sample is considered as a point in the multidimensional space. Point position is determined by coordinates that are cell values corresponding to the row of the table. Each variable (i.e., the column in the matrix data arrangement) indicates the coordinate of the axis in the given space.

Experimental data were also processed using partial least squares (PLS) regression in order to develop prediction models. The evaluation of the fit of the models was done by correlating the reference (calibration) values and the values calculated by the models of the prediction set. The calibration and validation data were selected to ensure that both datasets were representative of the experimental design. The accuracy of the models was expressed as the root mean square error of calibration (RMSE) and corresponding correlation coefficient both for calibration and prediction.

2.6. Coulometric Titration

The coulometric system Unicoulo ("Nightfly systems s.r.o.", Prague, Czech Republic) is designed for constant current coulometry with biamperometric indication. The measuring unit contains the appropriate interface board, a measuring board, a power supply, and a voltage and current source necessary for coulometric measurements, as well as indicator electrodes and a USB port to connect to computer [10]. The electrode system consists of a generating and indicating cell. The generating cell consists of two flat platinum electrodes: a working electrode and a counter electrode. The counter electrode is separated from the solution by a porous glass frit. The tube is filled with 0.5 mol L^{-1} sodium sulphate solution. The signal is recorded through two platinum indication electrodes, which are mounted in the same holder. The electrode system is compatible with Metrohm equipment (Metrohm, Herisau, Switzerland).

Determination of ascorbic acid content in commercial vitamins by coulometric titration is based on the oxidation of ascorbic acid by iodine (see Supplementary Materials). Coulometric titration is performed at a constant 10 mA generating current. First, the electrode system was electrochemically cleaned before the first measurement and also after each measurement for proper function when changing the polarity of the working electrode from positive to negative for 90 s. Then, the optimal polarization voltage at 200 mV was selected. A blank measurement followed. The blank value provides information on the content of impurities in chemicals. The base electrolyte (10 mL NaI, 10 mL oxalic acid, and H_2O) was mixed and the titration curve was measured using the optimal polarization potential [10]. The effervescent tablets dissolved in distilled water were processed according to the procedure given in Section 2.4.

3. Results and Discussion

3.1. Type of Polymericized Films for Electrode Array

The experimental potentiometric ET in this work was conceived as a flow system and was composed of four types of sensors. Each type of sensor was repeatedly prepared three times, and a total of 12 sensors were used (see Section 2.3). Polymers were deposited on Pt electrodes: poly (4-amino-2,1,3-benzothiadiazole) abbreviated PABTD, poly (3,4-diaminobenzoic acid) (PDABA), and poly (neutral red) (abbreviated PNR) and a bare Pt electrode served as a reference sensor. The polymerization processes of the individual monomers used in this work are described in detail in our previous studies [25–27], where electrochemical and spectroscopic characterizations of individual polymers were utilized. Briefly, we summarize here that the polymerization occurred (Supplementary Materials, see Figure S1) in accordance with the cited publications, and the individual redox peaks formed during the polymerization are indicated in the Figure S1a–c by the letters A (anodic peaks) and C (cathodic peaks). In all three cases, colored polymerized films were formed on the surface of the platinum wire electrodes, and were visible to the naked eye.

These oxidized conductive polymers and their derivatives are formed by a polycationic backbone and a charge-compensating anion. From the point of view of potentiometry, depending on the size and mobility of the incorporated ions, the conductive polymers act as ion exchangers of anions or cations and consequently give an anionic or cationic potentiometric response. The potentiometric response is also affected by electron transfer at the polymer–solution interface if redox components are present in the solution [23,24,29]. The potential response is then the result of a redox process that changes the redox composition of the polymer surface due to the equilibrium between the polymer and the solution. These three types of polymers were selected based on their selectivity and the sensitivity to different analytes described in previous studies [25–27]. PABTD-coated platinum electrodes are sensitive to divalent cations [26]. PNR electrodes show a selective response of citrates at pH 8.5 [27] and PNR is also a known redox mediator [30]. Modified PDABA electrodes are sensitive to sugars [25]. At the same time, it should be mentioned that all used polymer layers are very sensitive to pH change [29] and to redox substances such as dopamine [15–17,31], ascorbic acid [9,15–17], and others.

The experimental ET was set up to test samples of vitamin C effervescent tablets. These real samples contain, in addition to vitamin C, a number of other substances such as cations, citric acid, sugars, and fillers (Supplementary Materials, see Table S1), and therefore, it was assumed that selected sensors should be able to distinguish between various types of samples effervescent tablets.

3.2. Potentiometric Characterization of Electrode Array

3.2.1. Response to Ascorbic Acid and pH

Firstly, it was necessary to calibrate the ET, i.e., to test the response of the sensors to standard ascorbic acid (AA) solutions. The potentiometric response of the individual sensors to the concentration of AA dissolved in acetate buffer at pH 5.0 in the range of 10^{-6} to 10^{-1} mol L^{-1} was measured (see Figure 2a). The measurements showed that all types of electrodes (polymer and bare Pt) are sensitive to AA in the concentration range of 10^{-3} to 10^{-1} mol L^{-1} with a slope from −64.5 to −89.1 mV per concentration decade. The slope was higher than the theoretical one (−59.2 mV), due to the influence of the underlying platinum, which readily reacts to the presence of redox substances in the solution [22]. Electrodes modified with PNR and also PDABA in the range of 10^{-4} to 10^{-1} mol L^{-1} showed the best response to AA.

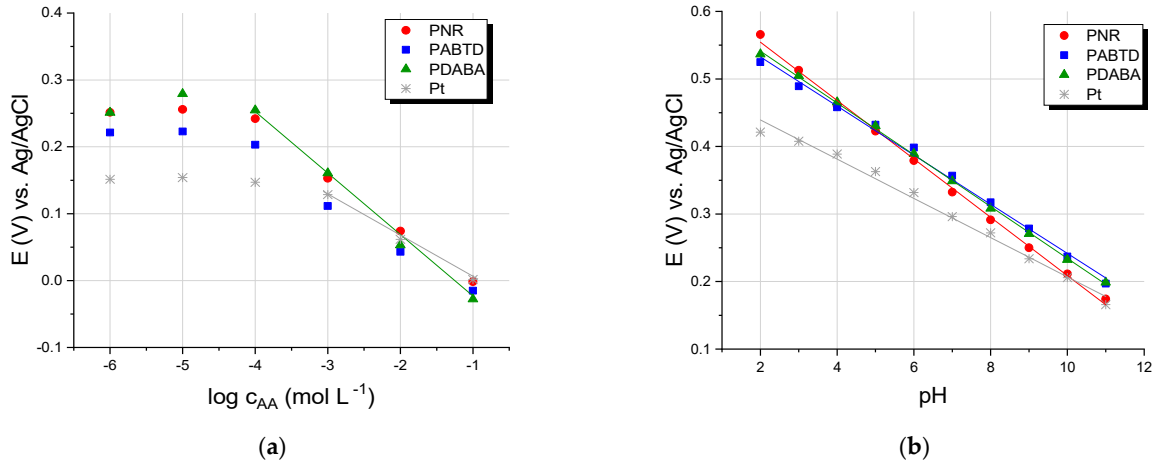


Figure 2. Dependences of electromotive voltage of individual types of electrodes (**a**) on the logarithm of the concentration of ascorbic acid (AA) dissolved in acetate buffer at pH 5, and (**b**) on the pH in Britton–Robinson buffer of pH range 2–11.

Furthermore, the response of the ET to the changes in pH values in Britton–Robinson buffer in the range of pH 2 to 11 was tested. Hydrogen ions can be involved in ion exchange process at the polymer–solution interface, where H^+ ions are preferred over others due

to their high mobility, or protonation/ deprotonation of the polymer film with a suitable acid [29]. It is clear from Figure 2b that all types of electrodes are sensitive to the changes in pH over almost the entire measured range. However, the bare Pt electrode shows the lowest slope (half value compared to the polymer electrodes), which has also been observed in other studies [22]. The highest slopes and the best pH responses are achieved by electrodes coated with PNR (−48.2 mV/decade) and PDABA (−41.3 mV/decade).

3.2.2. Stability of Potentiometric Signal

Stability is a key parameter in testing sensor arrays and plays an important role in the repeated recognition of model and real samples. Our previous studies showed that ageing affects the properties of polymer films as well as the whole recognition system. If platinum electrodes are used, the stability of the sensors is also affected by the previous treatment of the substrate material [22] or by measured medium [32]. Long-term stability of individual electrodes in the sensor array was tested during one and a half months.

The signal stability of individual electrodes was monitored during the analysis of real samples of effervescent tablets. The measurement was performed by inserting several washing solutions (6) with acetate buffer of pH 4 between the individual samples of effervescent tablets. This lower pH buffer was intended to wash the electrode surface from sample residues and possibly remove adsorbed substances from the electrode surface. The measurement revealed changes in the response of the electrodes, which are summarized in Figure 3. PNR electrodes showed a different trend compared to other types of electrodes and appear to be the most stable. On the contrary, the most fluctuating potential values were observed for PDABA electrodes, which may be related to structural changes of polymerized film in time or its gradual degradation during long-term measurements. The stability of the electrodes was evaluated in the following order: PNR (max) > PABTD > Pt > PDABA (min).

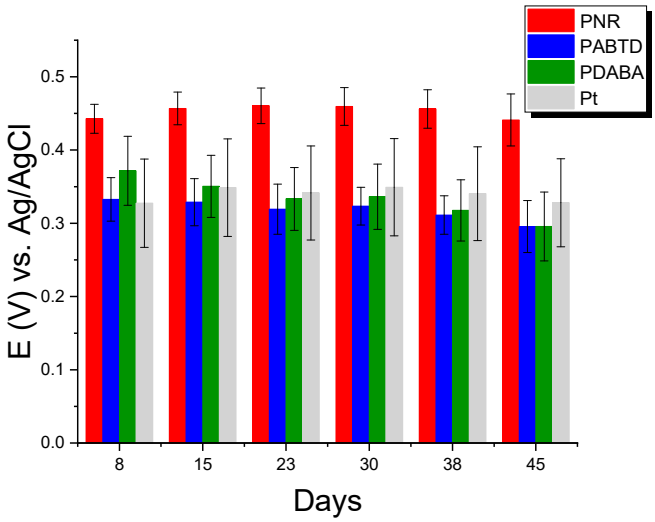


Figure 3. Potential response stability for all types of investigated electrodes over time, measured in acetate buffer pH 4.0, n = 3.

According to the literature, ascorbic acid is oxidized on platinum electrodes in a two-electron process [33,34]. There are two oxidation reaction pathways, one corresponding to the irreversible electrode reaction of the substance without appreciable adsorption and the other to the oxidation of the adsorbed substance in the potential extent of Pt oxide surface formation [34]. At the same time, oxidation of AA Pt electrode is accompanied by spontaneous adsorption of other molecules, e.g., CO [33]. In addition to CO, hydrogen [35] and oxygen [34] can also be sorbed from the solution onto the Pt surface, then various Pt oxides (PtO, PtO_2) are formed [35], which influence Pt behavior. Since more products can be formed on the surface of Pt during the oxidation of AA and sorbed easily and unevenly

on the surface, the behavior of pure Pt is different from that of polymer electrodes. The higher standard deviations of the Pt electrode compared to the polymerized electrodes are consistent with the statement that the surface of the Pt electrodes is highly heterogeneous, even though the electrodes are cleaned under the same conditions before measurement. This is apparent from Figure S2 in the Supplementary Materials, where irregularities and also possible defects (scratches) occur on the surface of Pt. On the other hand, the overlay surface layer of homogeneous polymer eliminates such non-uniformity and the signal of the polymerized electrodes is then reproducible.

3.3. Pattern Recognition of Effervescent Tablets Dissolved in Acetate Buffer Using an Electronic Tongue

3.3.1. Analysis/Measurements of Effervescent Tablets

Electronic tongue is used to distinguish real samples of drugs based on their different tastes. This is used in the manufacture of drugs to mask their unpleasant taste [36]. ET based on conductive layers can quickly recognize individual real samples of effervescent tablets based on (i) different vitamin C content, (ii) pH value, and (iii) different overall composition. After dissolving samples of vitamin C effervescent tablets in acetate buffer pH 5.0, the potential response of the electrode array was measured. The average values of the electromotive voltage of the individual electrodes of the electrode array (hereinafter the potential of the electrodes) immersed in the samples of effervescent tablets were evaluated. From Figure 4, it is obvious that the highest potential of the ET is achieved with the effervescent tablet C with the lowest content of AA. Conversely, the lowest potential was measured for the effervescent tablet E, which has the highest AA content. The potential of almost all types of electrodes decreases in the order of samples S_C > S_A > S_B > S_D > S_E. These results are completely consistent with the coulometric determination (see Table 4 and Table S2). The only exception is the polymer electrode PNR, which shows the highest potential in the effervescent tablet A instead of C, however, the other tablets follow the same trend S_B > S_D > S_E consistent with the other types of electrodes. This phenomenon may be related to the composition of tablets A and C, where the response of PNR electrodes is also influenced by the presence of other components such as citric acid or various sugars and dyes (Supplementary Materials, see Table S1). PDABA and PNR electrodes had the highest repeatability of potential measurement based on the lowest value of standard deviations. The values of the standard deviations are presented in Figure 4 by means of error bars.

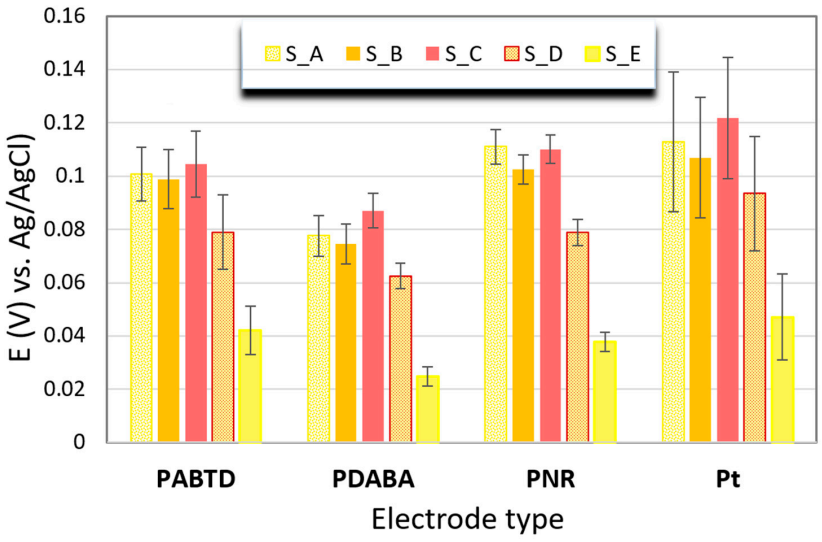


Figure 4. The potential response of the electrode array (12 electrodes) on 5 samples of effervescent tablets (labeled S_A–S_E) dissolved in acetate buffer. The color scale of the columns corresponds to the actual color of the measured tablets. Error bars represent sampling standard deviations for n = 3.

Table 4. Comparison of ascorbic acid (AA) in effervescent tablet samples (labeled S_A–S_E) specified by the manufacturer on the packaging label and experimentally determined using coulometric titration for n = 6.

Effervescent Tablets	Producer	Concentration of AA in Tablet [mM] Dissolved in 100 mL Volume Flask	Amount of AA Declared by Manufacturer in Tablet [mg]	Amount of AA Obtained by Coulometric Titration in Tablet [mg] [1]	Difference in Declared Experimental Amount of AA [mg]	Deviation [%]
S_A	Haas	4.5	80	85.8 ± 0.9	5.8	7.3
S_B	Haas	4.5	80	87.1 ± 0.7	7.1	8.9
S_C	Haas	2.3	40	39.7 ± 0.8	0.3	0.8
S_D	MaxiVita	4.5	80	97.2 ± 1.7	17.2	21.5
S_E	MaxiVita	13.6	240	263.0 ± 1.7	23.0	9.6

[1] The confidence interval was calculated using the formula $u = (t_{n-1} \cdot s)/\sqrt{n}$, where t_{n-1} is the critical value of the Student's distribution for $(n - 1)$ degrees of freedom and the significance level $\alpha = 0.05$, n is the number of measured values, and s is the standard deviation.

3.3.2. PCA Processing of the Potentiometric Data—Recognition of Effervescent Tablets

Data obtained by potentiometric ET (Section 3.3.1), based on the sensor array consisting of 12 electrodes PABTD, PDABA, PNR, and Pt, were used for the analysis of PCA. The results of the PCA are presented in the form of a scattering diagram of the component score values, where average score values along the first (PC1) and the second (PC2) principal component are represented. Figure 5a,b distinguish effervescent tablets A–E (labeled from S_A to S_E) dissolved in acetate buffer or in distilled water according to their AA content. Incorporation of the points within the compact clusters (delimitation of the same samples S_A–S_E) indicates a very good repeatability of the sensor array measurements for similar samples. From Figure 5a,b, it is evident that the variability along the first component PC1 is crucial for the recognition of individual samples, covering 99% of the described overall variability. The distribution of clusters with respect to the horizontal axis (PC1) is therefore significant. The higher and lower values of the measured electrode potential correspond to the distribution of data sets along positive or negative PC values. Based on the distribution of clusters, we can conclude that tablets with a higher content of ascorbic acid, namely tablet E (S_E) (8.4%) and tablet D (3.8%), can be easily distinguished and separated from tablets A, B, and C (S_A–S_C), which in turn have the lowest content of this vitamin (S_A–S_C: 2.1%, 2.2%, and 1.0%). It has to be noted that due to the very similar contents in tablets A, B, and C, there is only a slight difference in the positions of the individual clusters along the PC1, yet they can be well distinguished and do not overlap each other (see Figure 5a). From this point of view, even the slight distribution of variability change along PC2 is important. Comparing to acetate buffer solution, when using distilled water as a tablet solvent, the differences between the positions of the individual clusters representing samples A, B, C, and D along PC1 are minimized, which leads to the fact that their resolution is worse than in the case of acetate buffer; however, the clusters are separated without overlapping (see Figure 5b). Additionally, clusters of all data sets are less compact due to the higher variability change along the PC2. This difference in resolution of samples of effervescent tablets in water is due to the slight varieties in the pH values in addition to various content of vitamin C. The pH value of individual samples of effervescent tablets is given in the Table 2. Due to the better resolution of the individual effervescent tablets, the acetate buffer appears to be a more suitable environment for the analysis of these samples by a proposed electronic tongue.

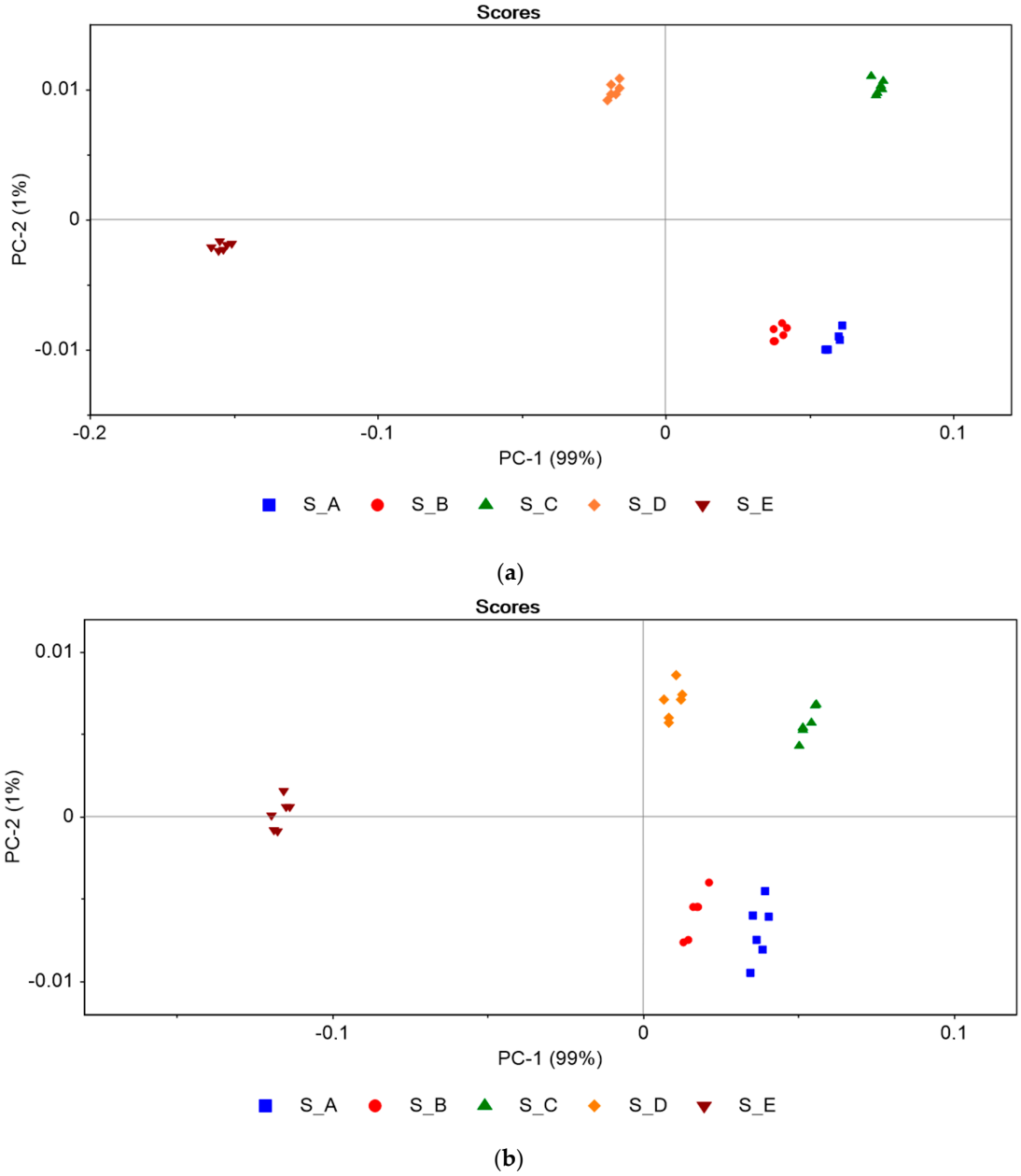


Figure 5. Scattering plot of the component score values for the PCA method of samples of effervescent tablets S_A–S_E dissolved (**a**) in acetate buffer and (**b**) in distilled water. Each sample was measured 6 times and each point on the graph corresponds to the average value of 3 measurements.

3.3.3. Prediction Models

Data from potentiometric ET and coulometric titration were used for the development of PLS method. The calibration model (see Figure 6), well captures the relationship between the measured signal (electrode potential) and the determined concentration (vitamin C content in effervescent tablets determined by coulometric titration). In the developed regression models, the differences between actual and calculated values of AA concentration were minimal. Both established models reached good values of the statistical parameters. The model obtained for the data in acetate buffer accurately captures the concentration dependence, i.e., the predicted concentration value agrees with the reference value (Figure 6). The direction of the line (Slope) in Figure 6 is 0.9993 and the value of the correlation coefficient (R-Square) is 0.9996; values of calibration and prediction errors are also corresponding.

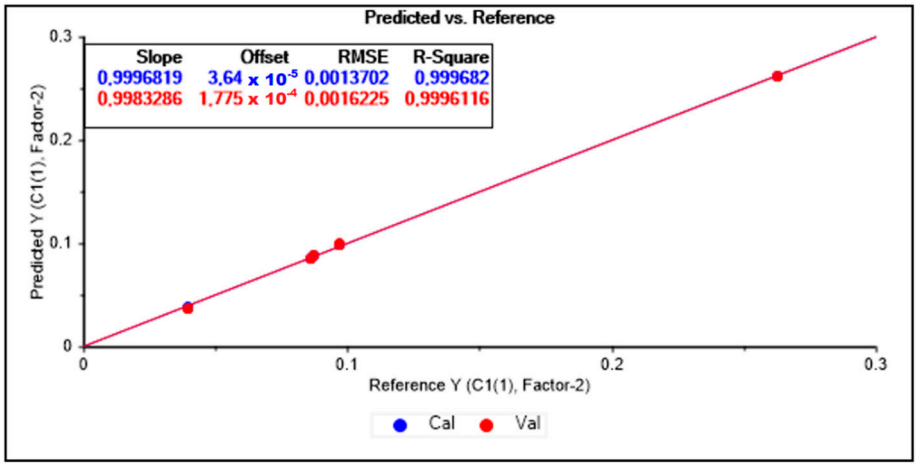


Figure 6. Correlation dependence of reference (coulometric titration) and predicted concentration (electrode potential) obtained by PLS method for effervescent tablets dissolved in acetate buffer.

The detailed results of the determination of vitamin C content in effervescent tablets obtained from coulometric titration are described in Supplementary Materials (see Tables S1 and S2, and Figure S3). The ascorbic acid content of the individual tablets declared by the manufacturer on the packaging and the experimentally determined values obtained by coulometric titration are summarized in Table 4. Excellent agreement between the results obtained from experimental measurements, and data from the manufacturer, was achieved in tablet C, with the lowest content of ascorbic acid. Tablets A, B, and E show relatively identical results (up to 10%). Conversely, the largest difference was observed in tablet D, where the contents differ by up to about 17 mg, representing about 22%.

To our best knowledge of the literature, there is only a few articles on the application of ET for the potentiometric detection of AA and almost none with electropolymerized film electrodes. The literature results of the determination of AA content in different samples obtained from potentiometric and voltammetric ET are summarized in Table 5.

Table 5. Summarization of others electronic tongue sensors for AA detection.

Electronic Tongue	Electrode	Layer/ Receptor	Statistical Method	Analyte	Concentration (mol L^{-1})	Real Sample	Reference
Potentiometric ET	8: miniaturized PVC membrane electrodes	an ion-exchanger	PLS	**AA**, acetylsalicylic acid, acetaminophen	$10^{-5.5}$–$10^{-1.5}$	quantitative analysis of mixtures, no real samples	[37]
Voltammetric ET	3: Pt, Au, epoxy-graphite	–	ANN	**AA**, uric acid, acetaminophen (paracetamol)	10^{-4}–10^{-3}	50 standard solutions, mixture, no real sample	[38]
Voltammetric ET	4: ITO	phthalocyanine films (LB or LS technique)	PCA	**AA**, vannilic acid, pyrogallol, catechin	10^{-3}	mixture solutions, no real sample	[39]
Voltammetric ET	7: graphite epoxy composite electrodes or 8: Pt disc	cobalt (II) phthalocyanine, polypyrrole, Prussian blue, 4 different oxide nanoparticles	PCA CVA ANN	**AA**, paracetamol uric acid	10^{-4}	mixture solutions	[40]
Our potentiometric ET	12: Pt	Electro-polymerized films	PCA	**AA**	$10^{-6.1}$–$10^{-4.3}$	evanescent tablets	–

4. Conclusions

The potentiometric sensitivity of polymerized films derived from 4-amino-2,1,3-benzothiadiazole, 3,4-diaminobenzoic acid, and neutral red deposited on the platinum electrode surface to ascorbic acid was applied to assemble an experimental potentiometric electronic tongue. The stability of the electrode response depended on the type of polymerized film and varied in the following sequence: PNR (max) > PABTD > Pt > PDABA (min). The relationship between the potentiometric signal and the AA concentration allowed to identify effervescent tablets with different vitamin C contents. The difference observed in ET was in good agreement with the predicted model based on the PLS method and coulometric titration data. These results demonstrate the practicability of laboratory electronic tongue for the rapid determination of ascorbic acid in commercial samples; its further development and extension of applications to other commercial samples is offered.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/chemosensors9050110/s1, Figure S1: Cyclic voltammograms of polymerization of monomers; (a) NR, (b) ABTD, and c) DABA. The individual figures include curves of supporting electrolytes (dashed line). The detailed conditions of polymerization process are summarized in the Table 1, Figure S2: Dependence of the indication current on the time in the blank experiment (Bl) and in the analysis of samples of effervescent tablets A–E (marked S_A–S_E), Table S1: Composition of effervescent tablets (labeled S_A–S_E), Table S2: Measured and calculated data of coulometric titration of ascorbic acid (AA) in effervescent tablet samples (labeled S_A–S_E) for n = 6. The volume of 1 mL was pipetted to the cell.

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References

1. Ciosek, P.; Wroblewski, W. Sensor arrays for liquid sensing—Electronic tongue systems. *Analyst* **2007**, *132*, 963–978. [CrossRef]
2. Woertz, K.; Tissen, C.; Kleinebudde, P.; Breitkreutz, J. Taste sensing systems (electronic tongues) for pharmaceutical applications. *Int. J. Pharm.* **2011**, *417*, 256–271. [CrossRef] [PubMed]
3. Kobayashi, Y.; Habara, M.; Ikezazki, H.; Chen, R.; Naito, Y.; Toko, K. Advanced taste sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores. *Sensors* **2010**, *10*, 3411–3443. [CrossRef] [PubMed]
4. Krejzová, E.; Bělohlav, Z. Masking the Taste of Active Substances in Orally Dispersible Tablets. *Chem. Listy* **2004**, *108*, 17–24.
5. Sohi, H.; Sultana, Y.; Khar, R.K. Taste masking technologies in oral pharmaceuticals: Recent developments and approaches. *Drug Dev. Ind. Pharm.* **2004**, *30*, 429–448. [CrossRef] [PubMed]
6. Ayenew, Z.; Puri, V.; Kumar, L.; Bansal, A.K. Trends in pharmaceutical taste masking technologies: A patent review. *Recent Pat. Drug Deliv. Formul.* **2009**, *3*, 26–39. [CrossRef]
7. Wagh, D.V.; Ghadlinge, S.V. Taste Masking Methods and Techniques in Oral Pharmaceuticals: Current Perspectives. *J. Pharm. Res.* **2009**, *2*, 1049–1054.
8. Shishkanova, T.V.; Broncová, G.; Skálová, A.; Prokopec, V.; Člupek, M.; Král, V. Potentiometric electronic tongue for taste assessment of ibuprofen based pharmaceuticals. *Electroanalysis* **2019**, *31*, 2024–2031. [CrossRef]
9. Tsai, M.-H.; Lu, S.H.; Lai, Y.H.; Lai, G.-H.; Dizon, G.V.; Yang, T.I.; Lin, Y.-J.; Chou, Y.C. Novel ascorbic acid sensor prepared from gold/aniline-pentamer-based electroactive polyamide composites. *Express Pol. Lett.* **2018**, *12*, 71–81. [CrossRef]
10. Broncová, G.; Řezanka, P.; Vosmanská, M.; Shishkanova, T.V. List of Laboratory Task, UCT Prague Coulometry. 2019. Available online: https://uanlch.vscht.cz/studies/bachelor_en/laboratories_en/lachi_en/list_en (accessed on 1 January 2020).

11. Pisoschi, A.M.; Pop, A.; Serban, A.I.; Fafaneata, C. Electrochemical methods for ascorbic acid determination. *Electrochim. Acta* **2014**, *121*, 443–460. [CrossRef]
12. Abdullin, I.F.; Turova, E.N.; Ziyatdinova, G.K.; Budnikov, G.K. Potentiometric determination of ascorbic acid: Estimation of its contribution to the total antioxidant capacity of plant materials. *J. Anal. Chem.* **2002**, *57*, 353–355. [CrossRef]
13. Fernandes, J.C.B.; Kubota, L.T.; de Oliveira Neto, G. Potentiometric biosensor for l-ascorbic acid based on ascorbate oxidase of natural source immobilized on ethylene–vinylacetate membrane. *Anal. Chim. Acta* **1999**, *385*, 3–12. [CrossRef]
14. Luo, X.-L.; Xu, J.-J.; Zhao, W.; Chen, H.-Y. Ascorbic acid sensor based on ionsensitive field-effect transistor modified with MnO_2 nanoparticles. *Anal. Chim. Acta* **2004**, *512*, 57–61. [CrossRef]
15. Kalimuthu, P.; John, A.S. Electropolymerized film of functionalized thiadiazole on glassy carbon electrode for the simultaneous determination of ascorbic acid, dopamine and uric acid. *Bioelectrochemistry* **2009**, *77*, 13–18. [CrossRef] [PubMed]
16. Roy, P.R.; Okajima, T.; Ohsaka, T. Simultaneous electroanalysis of dopamine and ascorbic acid using poly(N-N′-dimethylaniline)-modified electrodes. *Bioelectrochemistry* **2003**, *59*, 11–19. [CrossRef]
17. Yao, H.; Sun, Y.; Lin, X.; Tang, Y.; Huang, L. Electrochemical characterization of poly(eriochrome black T) modified glassy carbon electrode and its application to simultaneous determination of dopamine, ascorbic acid and uric acid. *Electrochim. Acta* **2007**, *52*, 6165–6171. [CrossRef]
18. Vasantha, V.S.; Chen, S.-M. Electrocatalysis and simultaneous detection of dopamine and ascorbic acid using poly(3,4-ethylenedioxy)thiophene film modified electrodes. *J. Electroanal. Chem.* **2006**, *592*, 77–87. [CrossRef]
19. Riul, A.; Gallardo Soto, A.M.; Mello, S.V.; Bone, S.; Taylor, D.M.; Mattoso, L.H.C. An electronic tongue using polypyrrole and polyaniline. *Synth. Met.* **2003**, *132*, 109–116. [CrossRef]
20. Paolesse, R.; Lvova, L.; Nardis, S.; Di Natale, C.; D'Amico, A.; Castro, F.L. Chemical imagines by porphyrin arrays of sensors. *Microchim. Acta* **2008**, *163*, 103–112. [CrossRef]
21. Riul, A.; Malmegrim, R.R.; Fonseca, F.J.; Mattoso, L.H.C. An artificial taste sensor based on conducting polymers. *Biosens. Bioelectron.* **2003**, *18*, 1365–1369. [CrossRef]
22. Shishkanova, T.V.; Broncová, G.; Krondak, M.; Sýkora, D.; Král, V. Important aspects influencing stability of the electrochemical potential of conductive polymer-based electrodes. *J. Mater. Sci.* **2011**, *46*, 1–9. [CrossRef]
23. Maksymiuk, K.; Nybäck, A.-S.; Bobacka, J.; Ivaska, A.; Lewenstam, A. Metallic and non-metallic redox response of conducting polymers. *J. Electroanal. Chem.* **1997**, *430*, 243–252. [CrossRef]
24. Bobacka, J.; Ivaska, A.; Lewenstam, A. Potentiometric Ion Sensors Based on Conducting Polymers. *Electroanalysis* **2003**, *15*, 366–374. [CrossRef]
25. Broncová, G.; Shishkanova, T.V.; Dendisova, M.; Clupek, M.; Kubac, D.; Matejka, P. Poly(4-amino-2,1,3-benzothiadiazole) films: Preparation, characterization and applications. *Chem. Pap.* **2017**, *71*, 359–366. [CrossRef]
26. Broncová, G.; Matějka, P.; Němečková, Z.; Vrkoslav, V.; Shishkanova, T.V. Electrochemical detection of sialic acid using phenylboronic acid-modified poly(diaminobenzoic) acid. *Electroanalysis* **2018**, *30*, 672–680. [CrossRef]
27. 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. [CrossRef]
28. Březnová, 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. [CrossRef] [PubMed]
29. Broncová, G.; Shishkanova, T.V.; Krondak, M.; Volf, R.; Král, V. Potentiometric Sensors Based on Conducting Polymers: Preparation, Response Mechanisms and Applications. *Chem. Listy* **2009**, *103*, 795–799.
30. Karyakin, A.A.; Ivanova, Y.N.; Karyakina, E.E. Equilibrium (NADţ/NADH) potential on poly(Neutral Red) modified electrode. *Electrochem. Commun.* **2003**, *5*, 677–680. [CrossRef]
31. Shaidarova, L.G.; Gedmina, A.V.; Artamonova, M.L.; Chelnokova, I.A.; Budnikov, H.C. Voltammetry determination of dopamine by the electrocatalytic response of an electrode modified by a polyaniline film with an inclusion of copper(II) tetrasulfophthalocyanine. *J. Anal. Chem.* **2013**, *68*, 516–524. [CrossRef]
32. Broncová, G.; Shishkanova, T.V.; Krondak, M.; Volf, R.; Král, V. Optimalization of Poly(neutral red) Coated-wire Electrode for Determination of Citrate in Soft Drinks. *Sensors* **2008**, *8*, 594–606. [CrossRef]
33. Xing, X.; Bae, I.T.; Shao, M.; Liu, C.C. Electra-oxidation of L-ascorbic acid on platinum in acid solutions: An in-situ FTIRRAS study. *J. Electroanal. Chem.* **1993**, *346*, 309–321. [CrossRef]
34. Brezina, M.; Koryta, J.; Loucka, T.; Marsikova, D.; Pradac, J. Adsorption and kinetics of oxidation of ascorbic acid at platinum electrodes. *J. Electroanal. Chem.* **1972**, *40*, 13–17. [CrossRef]
35. Thacker, R.; Hoare, J.P. Sorption of oxygen from solution by noble metals: I. Bright platinum. *J. Electroanal. Chem. Interf. Electrochem.* **1971**, *30*, 1–14. [CrossRef]
36. Newman, J.; O'Riordan, D.; Jacquier, J.-C.; O'Sullivan, M. Masking of bitterness in dairy protein hydrolysates: Comparison of an electronic tongue and a trained sensory panel as means of directing the masking strategy. *LWT Food Sci. Technol.* **2015**, *63*, 751–757. [CrossRef]
37. Wesoły, M.; Cetó, X.; del Valle, M.; Ciosek, P.; Wróblewski, W. Quantitative Analysis of Active Pharmaceutical Ingredients (APIs) Using a Potentiometric Electronic Tongue in a SIA Flow System. *Electroanalysis* **2016**, *28*, 626–632. [CrossRef]
38. Gutés, A.; Calvo, D.; Cespedes, F.; del Valle, M. Automatic sequential injection analysis electronic tongue with integrated reference electrode for the determination of ascorbic acid, uric acid and paracetamol. *Microchim. Acta* **2007**, *157*, 1–6. [CrossRef]

39. Casilli, S.; De Luca, M.; Apetrei, C.; Parra, V.; Arrieta, Á.A.; Valli, L.; Jiang, J.; Rodríguez-Méndez, M.L.; De Saja, J.A. Langmuir–Blodgett and Langmuir–Schaefer films of homoleptic and heteroleptic phthalocyanine complexes as voltammetric sensors: Applications to the study of antioxidants. *Appl. Surf. Sci.* **2005**, *246*, 304–312. [CrossRef]
40. Sarma, M.; Romero, N.; Cetó, X.; del Valle, M. Optimization of Sensors to be Used in a Voltammetric Electronic Tongue Based on Clustering Metrics. *Sensors* **2020**, *20*, 4798. [CrossRef]