

Determination of benzoate in cranberry and lingonberry using solid-contact ion-selective electrode; comparison with ion chromatography



Åbo Akademi University

Faculty of Science and Engineering

Sajana Manandhar



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Supervisor: Ville Yrjänä (Åbo Akademi University)

Co-supervisors: Prof. Johan Bobacka (Åbo Akademi University)

Prof. Ivo Leito (University of Tartu)

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Abstract

The purpose of this thesis was to develop the solid-contact ion-selective electrodes and to characterize them as benzoate-selective electrodes. The developed electrodes were then applied for the determination of benzoate content in cranberry and lingonberry. The monomer 3,4-ethylenedioxythiophene (EDOT) was polymerized galvanostatically in the presence of chloride (Cl^-) to form a solid contact made of poly(3,4-ethylenedioxythiophene) (PEDOT) doped with chloride over the glassy carbon (GC) electrodes. An acyclic derivative of 1,3-bis(carbazolyl)urea was used as an ionophore. The PEDOT: Cl^- films on the GC electrodes were coated with either a control membrane or a benzoate-selective membrane by drop-casting a membrane cocktail. The electrodes were calibrated in sodium benzoate solution with and without 0.01 M phosphate buffer. The performance of the fresh electrodes was compared with that of the two-month-old electrodes. The slope of the electrodes changed from Nernstian to near-Nernstian as they aged by two months. The linearity range remained the same with minimal degradation in the limit of detection (LOD). The influence of phosphate on the electrode performance was also studied, which showed significant degradation of the LOD of the benzoate-selective electrodes. The selectivity of the electrodes over different anions was evaluated. The benzoate content in berries was determined potentiometrically by using the standard addition method for quantification. Similarly, the ion chromatography (IC) method was also used for the determination of benzoate. The amount of benzoate found potentiometrically was slightly higher than that determined by IC. The selectivity of the electrodes towards other anions besides benzoate might have resulted in higher quantification by the electrodes. However, the amount determined by both methods was comparable, suggesting the practical applicability of the developed electrodes for benzoate determination.

Keywords: solid-state ion-selective electrode; benzoate; an acyclic ionophore; ion chromatography

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Abbreviations

The following abbreviations are used in this thesis.

CWE	Coated-wire electrode
DOS	Dioctyl sebacate
EIS	Electrochemical impedance spectroscopy
GC	Glassy carbon
IC	Ion chromatography
ISEs	Ion-selective electrodes
LOD	Limit of detection
OCP	Open circuit potential
<i>o</i> -NPOE	2-nitrophenyl octyl ether
PEDOT	Poly(3,4-ethylenedioxythiophene)
PVC	Poly(vinyl chloride)
RMS	Root mean square
SC-ISEs	Solid-contact ion-selective electrodes
TDMACl	Tridodecylmethylammonium chloride
THF	Tetrahydrofuran

1 Introduction

Preservatives are additives that extend the shelf life of products by preventing or inhibiting spoilage caused by chemical reactions or microbiological activities. Benzoic acid and its salts are commonly used as preservatives and have a broad spectrum of antimicrobial activity against many bacteria, fungi, and yeasts¹. Benzoate salts are more soluble in water than benzoic acid. However, the undissociated benzoic acid is more effective as a preservative and benzoates exert their preservative action in acidic conditions. They are used as preservatives in food, beverages, cosmetics, and pharmaceuticals and the amount is regulated². The European Union (EU) has permitted benzoic acid (C_6H_5COOH) and its salts (E210-E213) as food additives with maximum permitted levels (MPLs) ranging from 150 to 6000 mg/kg³. The United States Food and Drug Administration (FDA) lists benzoic acid and sodium benzoate (C_6H_5COONa) as generally recognized as safe (GRAS) substances and the current maximum allowed level in food is 0.1%⁴. Benzoic acid can be obtained synthetically or naturally from some fruits, vegetables, nuts, spices, and dairy products. In plants, the concentration of benzoic acid depends upon species, climate, and geographical location⁵.

Berries are one of the natural sources of benzoic acid⁶. They have high amounts of organic acids, antioxidants, fibers, and minerals⁷. Berry juices are acidic due to the high amounts of organic acids. These are mainly malic acid, citric acid, and small amounts of benzoic acid. It is claimed that berries like lingonberries and cranberries contain enough benzoic acid for preservative action. A study by Viljakainen *et al.*⁸, showed that the amount of benzoic acid in the juices of some selected Nordic berries ranges from 0.1 to 0.7 g/l. The amount present is enough to inhibit both bacterial activity and yeast fermentation⁹, which makes the production of wine from berries challenging. Thus, it is useful to know the amount of benzoic acid present in berries. Since it is also used as an additive in various products, it is important to monitor the amount in them.

The most common analytical methods of choice for the determination of benzoic acid and sodium benzoate have been chromatographic methods such as reversed-phase high-performance liquid chromatography¹⁰⁻¹³, ion chromatography (IC)¹⁴, gas chromatography¹⁵⁻¹⁷, thin layer chromatography¹⁸. Other methods such as spectrophotometry¹⁹ and capillary electrophoresis²⁰ have also been reported. Most of these methods involve an extensive extraction procedure, which is time-consuming and labor-intensive or requires expensive equipment. Thus, it would be advantageous to develop a rapid, simple, and cheaper alternative

method for benzoate determination. Potentiometric ion sensors are well-known analytical devices for the determination of ions. They are easier and cheaper to fabricate and yet provide reasonable selectivity, sensitivity, and quick response²¹. Ion-selective electrodes (ISEs) are an important subgroup of potentiometric sensors²¹.

The main objective of this thesis is to develop a solid-contact benzoate-selective electrode for the determination of naturally occurring benzoate in berries. We also have compared its performance with ion chromatography. We have selected cranberry (*Vaccinium oxycoccus*) and lingonberry (*Vaccinium vitis-idaea*), which are commonly grown in Finland, as our sample. Along with the ISEs, we have also used an IC method as a standard method for the determination of benzoate in our samples. IC is a popular method used for the qualitative and quantitative determination of cations and anions. IC has been used in many analytical chemistry setups and has become a powerful and versatile technique. It has a broad range of applications with good accuracy, precision, and selectivity²². Further, we have compared two IC methods for better separation of benzoate from other anions, mainly malate and citrate that are present in higher amounts in berries. Moreover, we have also tried to show the possibility to apply the developed ISEs on real samples.

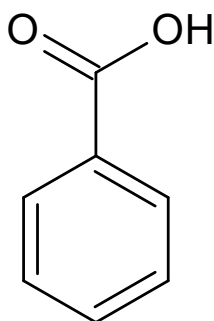


Figure 1. Benzoic acid

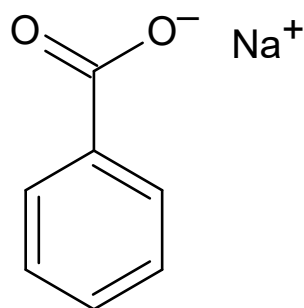


Figure 2. Sodium benzoate

2 Chemical sensors

Sensors are devices that provide a measurable signal by responding to a particular property of the substance. Sensors are of two major types: physical and chemical sensors. The former respond to a physical property like temperature, pressure, force, and magnetic field. The latter respond to a specific chemical species and produce a response that can be used for the qualitative or the quantitative determination of the species. All chemical sensors consist of (i) a chemical recognition layer that responds to a chemical species and produces a response and

(ii) a transducer that converts the response into a measurable signal. This response can be for example a change in color, mass, potential, or current. The response is proportional to the amount of analyte present in the sample. There are mainly four types of chemical sensors based on their mode of transduction namely electrochemical, optical, mass, and thermal sensors²³.

3 Ion-selective electrodes

ISEs are electrochemical sensors that allow the potentiometric determination of the activity of particular ions in the presence of other ions. The measured potential is proportional to the logarithm of the activity of the analyte ion in the sample solution. ISEs are very attractive for routine analytical applications as they are portable, small in size, economical, easy to use, and robust²⁴. With ISEs, it is possible to analyze samples with little to no sample pre-treatment and the measurement does not destroy the sample. The advancement of ISEs has made it possible to monitor the analyte with a wide dynamic range (typically 10^{-1} M to 10^{-6} M)²⁵⁻²⁸ and a lower detection limit as low as 10^{-11} M has been reported²⁹.

The development of ISEs began with the discovery of glass membrane sensitivity to hydrogen ions by Cremer³⁰. Haber and Klemensiewicz developed the first pH electrode based on glass³⁰. The pH electrode has been adopted as a routine analytical tool from 1936³¹. The progress made both in measuring techniques and the development of the special glasses resulted in the development of glass electrodes sensitive towards other cations (Na^+ , K^+ , Ag^+ , etc)³⁰. The next useful development in ISEs was a fluoride electrode based on europium-doped single-crystal lanthanum fluoride by Frant and Ross³². This electrode had a very high selectivity towards fluoride. The innovation by Simon *et al.* of a potassium-selective electrode using neutral ionophore in a plasticized membrane led to the development of ionophore-based ISEs³³. The pioneering work by Shirakawa *et al.* on polyacetylene attracted more interest towards conducting polymers³⁴. This essentially led to the path of innovative discovery of solid-contact ISEs based on conducting polymers³⁵.

4 Solid-contact ion-selective electrodes

Conventional ISEs are based on liquid contacts (Figure 3a). These electrodes consist of an inner liquid filling solution that functions as an ion-to-electron transducer between the ion-selective membrane (ISM) and the inner reference electrode. Besides them being highly reproducible and common in the market, they possess some problems, such as difficulty in controlling and

maintaining the level of internal filling solution and the electrode must always be in the upright position or at a slight angle so that the ISM is in contact with the filling solution. The use of conventional ISEs is inconvenient when the sample volume is small (e.g. clinical analysis) and the miniaturization of the liquid-contact ISEs is problematic³⁶. Eventually, the total elimination of inner filling solutions initiated the discovery of solid-contact ion-selective electrodes (SC-ISEs). The first SC-ISE, a coated-wire electrode (CWE) (Figure 3b), was developed by Cattrell and Freiser³⁷. CWEs are simple in design, cheaper, and are better suited to miniaturization. It was, however, difficult to achieve long-term potential stability with CWEs due to high charge-transfer resistance between the ISM and the electronic conductor³⁸. The need for well-defined ion-to-electron transduction between the ISM and the electronic substrate spurred on the development of SC-ISEs over the years. The introduction of the conducting polymers as solid contacts in the SC-ISEs (Figure 3c) was an undeniable breakthrough in the field of ion-selective electrodes. Conducting polymers significantly improved the potential stability and reproducibility which also enabled flexible structures and further miniaturization^{35,39}. Conducting polymers exhibit both ionic and electronic conductivity and serve as an intermediate contact or transducer between an ISM and an electronically conducting substrate.

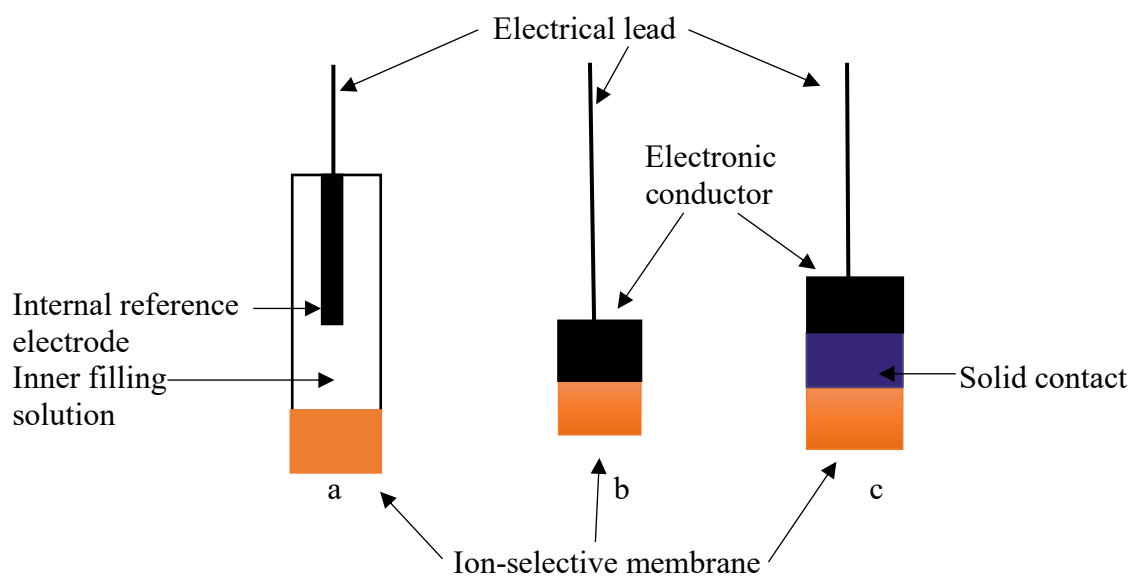


Figure 3. Schematic structure of a) a conventional ion-selective electrode, b) a coated wire electrode, and c) a solid-contact ion-selective electrode.

5 Composition of ion-selective membranes

Selectivity for the target analyte is achieved with the ISM. Several types of ISMs have been developed so far, for example, glass⁴⁰, crystal⁴¹, liquid membrane⁴², and polymeric membrane^{25,43,44}. Polymeric membrane-based ISMs are the most popular as they are versatile. ISMs have been developed for about 60 different analytes including inorganic cations and anions, neutral analytes, and organic ionic analytes⁴⁵. Normally high-molecular-weight poly(vinylchloride) (PVC) is used as a polymeric matrix. The polymer-based ISM is typically composed of a plasticizer, PVC, an ion-selective molecule (ionophore), and a lipophilic ion exchanger. A common ISM composition is approximately 66% plasticizer, 33% PVC, 1% ionophore, and a lipophilic ion exchanger.

An ionophore is a charged or neutral receptor that selectively binds to the analyte. It is expected that the lipophilicity of the ionophore retains the ionophore in the membrane. However, ionophores can also be covalently immobilized in the plasticized ISM⁴⁵. The ions might specifically fit into the cavity of the ionophore or form a bond with a functional group of the ionophore. A carbazole-derived acyclic receptor (Figure 4) has been used as an ionophore for benzoate anions in this work. The same ionophore had also been used for developing solid-contact acetate sensors previously. The electrodes showed modest selectivity to acetate and good selectivity to benzoate⁴⁶. Thus, in this study, we were interested in using this ionophore for developing benzoate sensors for real samples.

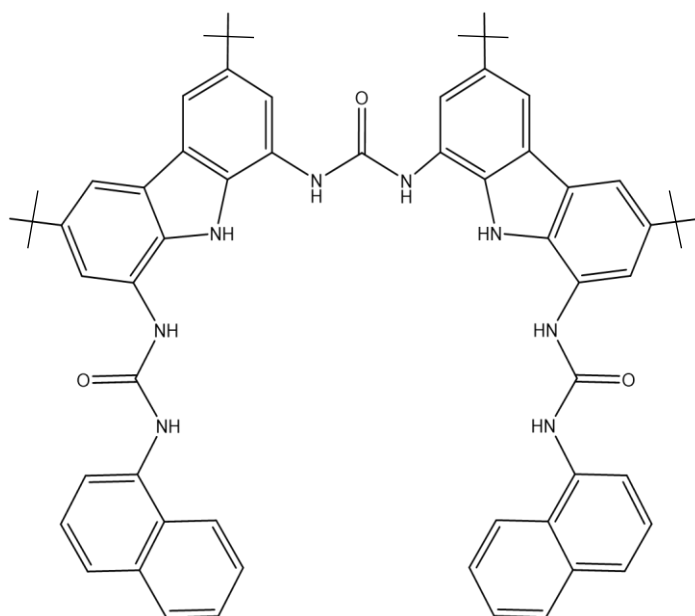


Figure 4. The ionophore, which is an acyclic derivative of 1,3-bis(carbazolyl)urea, that was used in this study.

PVC provides hydrophobicity to the membrane. Many plasticizers are highly viscous liquids at ambient temperature and are added to the PVC matrix to improve the flexibility of the membrane. They also serve as solvents for the ionophore. The dielectric constant of the plasticizers influences the selectivity coefficients ($K_{i,j}$) of the polymeric membrane⁴⁷. Leaching of plasticizers from the membrane is a common problem that limits the lifetime of membrane-based sensors. A highly lipophilic plasticizer has low leaching potential. Commonly used plasticizers include dioctyl sebacate (DOS) and 2-nitrophenyl octyl ether (*o*-NPOE).

Lipophilic cation or anion exchangers are added to the ionophore-based ISMs for the prevention of interferences from counterions and to create permselectivity. The addition of the ionic exchangers also reduces the response time and lowers the membrane resistance. However, the amount of exchanger added must be strictly limited by optimizing the molar ratio of lipophilic ion exchanger to ionophore; otherwise, the selectivity of the membrane can be changed drastically⁴⁸⁻⁵⁰. Potassium tetrakis(4-chlorophenyl)borate and potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate are commonly used as lipophilic cationic exchangers and tridodecylmethylammonium chloride is commonly used as lipophilic anionic exchanger.

6 Electrically conducting polymers

Electrically conducting polymers are the subject of interest and research owing to the advances in various applications such as sensors, solar cells, and organic light-emitting diodes. Conducting polymers in SC-ISEs are carbon-based polyene polymers. They have conjugated double bonds, i.e. alternating double bonds or π -conjugated systems. Conducting polymers are not capable of conduction in their pristine form, so for conduction, an electron/hole deficiency needs to be created. This can be done by a process called doping. Doping results in the delocalization of an electron or hole that serves as a charge carrier and induces conductivity in the conducting polymers. Doping can be done chemically or electrochemically. Doping with electron-deficient dopants or by oxidation is p-type doping. Reduction or electron-donating dopants induce n-type doping. Doping of an electrically conducting polymer increases the conductivity by tens to hundreds of siemens per cm^{51} . Polyaniline, polythiophene, and polypyrrole are the most common conducting polymers employed in ISEs.

Conducting polymers can be synthesized by chemical or electrochemical polymerization. Chemical polymerization is usually preferred when a large quantity of polymer is required. Polymers are formed by loss of chemical species from the reaction of the end group of

monomer chains in the presence of a chemical oxidant such as I_2 , Br_2 , $FeCl_3$, etc., and usually require a catalyst⁵². Electrochemical polymerization can be oxidative or reductive. It is a cost-effective and easy-to-use method. It is based on the deposition of the polymer on the surface of a solid electrode material like glassy carbon or metal. Oxidative electrochemical polymerization is the most suitable and commonly used method for polymerization. Generally, electrochemical polymerization can be achieved galvanostatically, potentiostatically, or potentiodynamically. In the galvanostatic mode of polymerization, a constant current is applied between the working electrode and the counter electrode that are dipped into the polymerization solution. The thickness of the deposited layer depends on the time of polymerization and the magnitude of current used. The potentiostatic technique can also be employed alternatively, which involves the application of a constant potential. The potential should be applied so that it will oxidize the monomer but avoid overoxidation or secondary reactions. The potentiodynamic technique can also be employed in which a varying potential is applied (e.g. cyclic voltammetry). The polymer is deposited on the electrode surface with the increasing number of cyclic scans. The thickness of the film also depends on the number of scans⁵³.

Poly(3,4-ethylenedioxythiophene), commonly known as PEDOT (Figure 5), has relatively high conductivity and remarkable environmental stability in its oxidized form. PEDOT has been extensively studied and employed in various applications such as ISEs, capacitors, solar cells, organic light-emitting diodes, and many more²⁴. We have used PEDOT as a solid contact in our ISE. Galvanostatic electrochemical synthesis was used for the polymerization of the ethylenedioxythiophene (EDOT) monomer over the GC electrode.

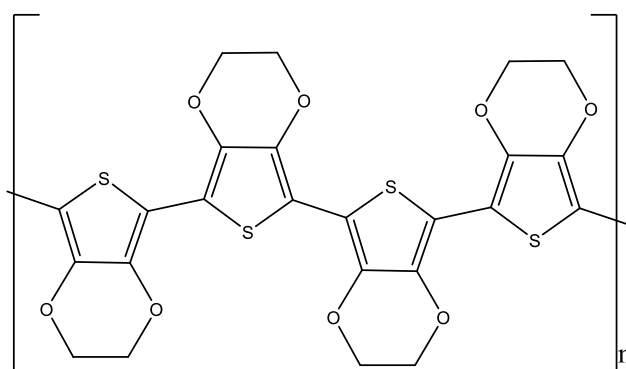


Figure 5. The chemical structure of poly(3,4-ethylenedioxythiophene) (PEDOT) in its undoped form.

7 Working principle

7.1 Potentiometry

The working mechanism of ISEs is based on the principle of potentiometry. The potential difference (electromotive force, EMF) between two electrodes, i.e., reference electrode and indicator electrode, is measured under zero current conditions using a high-impedance potentiometer. The ISE is the indicator electrode and the reference electrode has a constant potential. The EMF across the electrochemical cell is given by Equation 1:

$$EMF = E_{ind} - E_{ref} + E_j \quad \text{Equation 1}$$

where EMF is the potential difference measured, E_{ind} is the potential of the indicator electrode, E_{ref} is the potential of the reference electrode, and E_j is the liquid junction potential.

Ideally, all the potentials except the indicator potential should be sample-independent. The indicator potential depends on the target ion interaction with the ISM of the indicator electrode. A liquid junction potential is a potential that develops across the interface between two dissimilar solutions. The liquid junction potential depends on the charge, concentration, and mobility of ions. In the reference electrode, a junction potential develops between the inner reference solution and sample solution. Liquid junction potentials can be estimated using the Henderson equation⁵⁴:

$$E_j = \frac{\sum_i |z_i| U_i [C_i(\beta) - C_i(\alpha)]}{\sum_i |z_i| U_i [C_i(\beta) + C_i(\alpha)]} \frac{RT}{F} \ln \frac{\sum_i |z_i| U_i C_i(\alpha)}{\sum_i |z_i| U_i C_i(\beta)} \quad \text{Equation 2}$$

where z_i is the charge of ion i , U_i is its mobility, and C_i is its concentration in phase α (inner reference solution) and β (sample solution). The junction potential can be maintained at a low or constant potential, by using a high concentration of an electrolyte with cations and anions of fairly equal mobility, e.g. 3 M KCl.

In potentiometry, the electric potential developed between an ISE and a reference electrode immersed in the sample solution is linearly proportional to the logarithmic activity of the target ion. This relationship is described by the Nernst equation⁵⁵:

$$E = E^\circ + \frac{RT}{z_i F} \ln a_i \quad \text{Equation 3}$$

where E is observed potential, E° is the standard potential which depends on the characteristics of both indicator and reference electrode, R is the universal gas constant, T is the absolute temperature in Kelvin, F is the Faraday constant, z_i is the charge of the target ion i and a_i is its activity in the sample. The plot of observed potential against the logarithm of the activity of ion i should result in a calibration curve with a slope of $59.2/z_i$ mV/decade at 25 °C, which is positive for cations and negative for anions.

The selectivity of an ISE to a primary ion in presence of interfering ions in a solution can be determined by using the Nikolskii-Eisenman equation⁵⁶:

$$E = E^\circ + \frac{RT}{z_i F} \ln \left(a_i + \sum_j K_{i,j} a_j^{\frac{z_i}{z_j}} \right) \quad \text{Equation 4}$$

where z_i and z_j are the charges of the primary ion i and the interfering ion j , respectively, a_i and the a_j are the activities of the primary ion and the interfering ion, respectively. $K_{i,j}$ is the potentiometric selectivity coefficient of the ISE in presence of the interfering ion j . $K_{i,j}$ is an experimentally determined constant. The selectivity coefficient value of an ISE provides information on the numerical basis about its preference for the primary ion in presence of an interfering ion. A lower numerical value of $K_{i,j}$ of an ISE denotes higher discrimination of the interfering ion and preference for the primary ion. An ISE responds equally to the primary ion and the interfering ion if the selectivity coefficient is unity. The separate solution method and the fixed interference method are the recommended methods for the determination of the selectivity coefficient.

7.1.1 Standard addition methods for potentiometric measurements

Direct measurements are particularly useful for a sample with a simple matrix and for a specifically designed electrode for a specific ion. The standard addition method is useful in potentiometry when the sample matrix is complex, and the activity coefficients are difficult to predict. This method also overcomes the problem due to the partial complexation of the analyte³¹. This method is related to measuring the potential in a relatively large volume of sample and adding a smaller volume of the standard followed by the measurement of the resultant analyte signal. Single-addition and double-addition methods can be applied as standard addition methods in potentiometry.

It can be assumed that in a sample with an unknown activity coefficient, γ , only a fraction β of target analyte exists as free ions. The free target ion activity, α , in a sample is given by Equation 5:

$$\alpha = C^{ionized}\gamma = C^{total}\gamma\beta \quad \text{Equation 5}$$

The respective *EMF* value is

$$E_1 = E^0 + S \log \alpha^{initial} = E^0 + S \log C^{total} + S \log \gamma + S \log \beta \quad \text{Equation 6}$$

where S is the slope calculated from the calibration curve.

After the addition of a known amount of target analyte, ΔC^{total} , to the unknown sample, the *EMF* value is as follows.

$$E_2 = E^0 + S \log \alpha^{processed} = E^0 + S \log (C^{total} + \Delta C^{total}) + S \log \gamma + S \log \beta \quad \text{Equation 7}$$

The total concentration of the analyte in the native sample can be obtained by equation 8:

$$C^{total} = \frac{\Delta C^{total}}{10^{\frac{E_2 - E_1}{S}} - 1} \quad \text{Equation 8}$$

The added quantity of the target analyte must be such that the γ and β values remain constant. Therefore, a small quantity must be added. However, a small addition causes a small effect in the *EMF* and the accuracy of the result is low. So, the amount of target analyte added must be optimum and a few trials must be done to find the appropriate value of ΔC^{total} . This method is a single-addition method³¹.

The double-standard addition method can be used, when the response parameters in a complex sample may differ from those determined in single-electrolyte solutions. This method assumes that $C^{ionized} = C^{total} = C$. The *EMF* value is given by Equation 9:

$$E_1 = E^0 + S \log \gamma + S \log C \quad \text{Equation 9}$$

After the addition of ΔC of the analyte, the *EMF* is

$$E_2 = E^0 + S \log \gamma + S \log (C + \Delta C) \quad \text{Equation 10}$$

Then the same amount of analyte ΔC is added in the second addition and the *EMF* is

$$E_3 = E^0 + S \log \gamma + S \log (C + 2\Delta C) \quad \text{Equation 11}$$

Upon rearranging equations the final relation derived does not contain E^0 and S values.

$$\frac{E_3 - E_1}{E_2 - E_1} = \frac{\log \frac{C + 2\Delta C}{C}}{\log \frac{C + \Delta C}{C}} \quad \text{Equation 12}$$

Equation 12 can be solved by iteration procedure³¹.

7.2 Ion chromatography

Ion chromatography is a subtype of high-performance liquid chromatography in which the separation of the ions is based upon their interaction with ion-exchange resins and mobile phase. IC is an effective, reliable, and robust analytical tool for the analysis of inorganic ions and ionogenic organic species. It is a widely adopted analytical method for ionic analytes, since its discovery⁵⁷. The suppression-based ion chromatographic method, introduced by Small *et al.* in 1975, was a breakthrough in IC⁵⁸. The suppressor column was used to reduce the background conductivity of the eluent. Later, in 1979, Gjerde *et al.* developed a non-suppressed ion chromatographic technique, using low-capacity ion-exchange columns and low-conductive eluents^{59,60}. However, IC with the suppressed conductivity detection is most widely used. Conductivity detection is the common detection technique in IC, but the use of other detection systems like amperometric, spectrophotometric, refractive, chemiluminescence, and fluorescence in IC has enlarged the scope of application from inorganic ions to organic species like amino acids, nucleosides, proteins, carbohydrates, and glycols⁶¹. IC is routinely used in environmental analysis, clinical analysis, and in industries like pharmaceuticals, food, beverages, paper and pulps, metal processing and many more⁶².

Ion exchange is the mainstay separation mechanism in IC. Nonetheless, ionic analytes can be separated using other separation modes, such as ion exclusion⁶³ and ion pairing⁶⁴. Cations are separated using cation-exchange columns and anions are separated on anion-exchange columns. The stationary phase is made up of polymeric ion-exchange resins, such as polymethacrylate and polystyrene. Polar functional groups are attached to polymeric ion-exchange resins: e.g., sulfonate groups for a cation-exchange and quaternary ammonium groups for an anion-exchange resin. The counter ions are electrostatically attracted to the polar functional groups. Ion exchange occurs by replacing the counter ions from the ions in the sample solution, preserving electroneutrality. Acidic eluents are used for the separation of cations, while hydroxides or carbonates are used for the separation of anions. The separation occurs due to the competition of eluent ions and analyte ions for the electrostatic binding to the ionic functional group fixed to the column matrix. Separation is achieved as analytes migrate through the column at different rates depending on their degree of interaction to ion-exchange resins. The elution capacity of the eluent depends on its concentration, pH, ionic strength, temperature, and presence of organic solvents⁶⁵. In a suppression-based IC, the anion-exchange column is followed by a cation-exchange suppressor unit and *vice versa*. The suppression

mechanism removes the conductive ions from the eluent, thus reducing the background current. It also removes the counter ions in the sample and converts the analyte ion into a more conductive form, hence increasing the sensitivity. A schematic diagram of the suppression-based IC for the separation of cations and anions is shown in Figure 6. The ions are detected by the detector after passing through the suppression chamber.

7.2.1 Standard addition method for ion chromatography

The standard addition method is also the preferred method for quantification in chromatography for a sample with a complex matrix that interferes with analyte measurement signals⁶⁶. The sample solution is spiked with a known quantity of the substance to be determined. Experimentally, the standard addition can be performed by spiking varying known amounts of analyte to an equal amount of sample and diluting to the same volume. One sample is left unspiked. Concentration in the sample is calculated by extrapolating the regression line on the x-axis at which the y-intercept equals zero. The resulting negative x-intercept corresponds to the amount of analyte in the sample. However, this method is more labor-intensive than the calibration graph method.

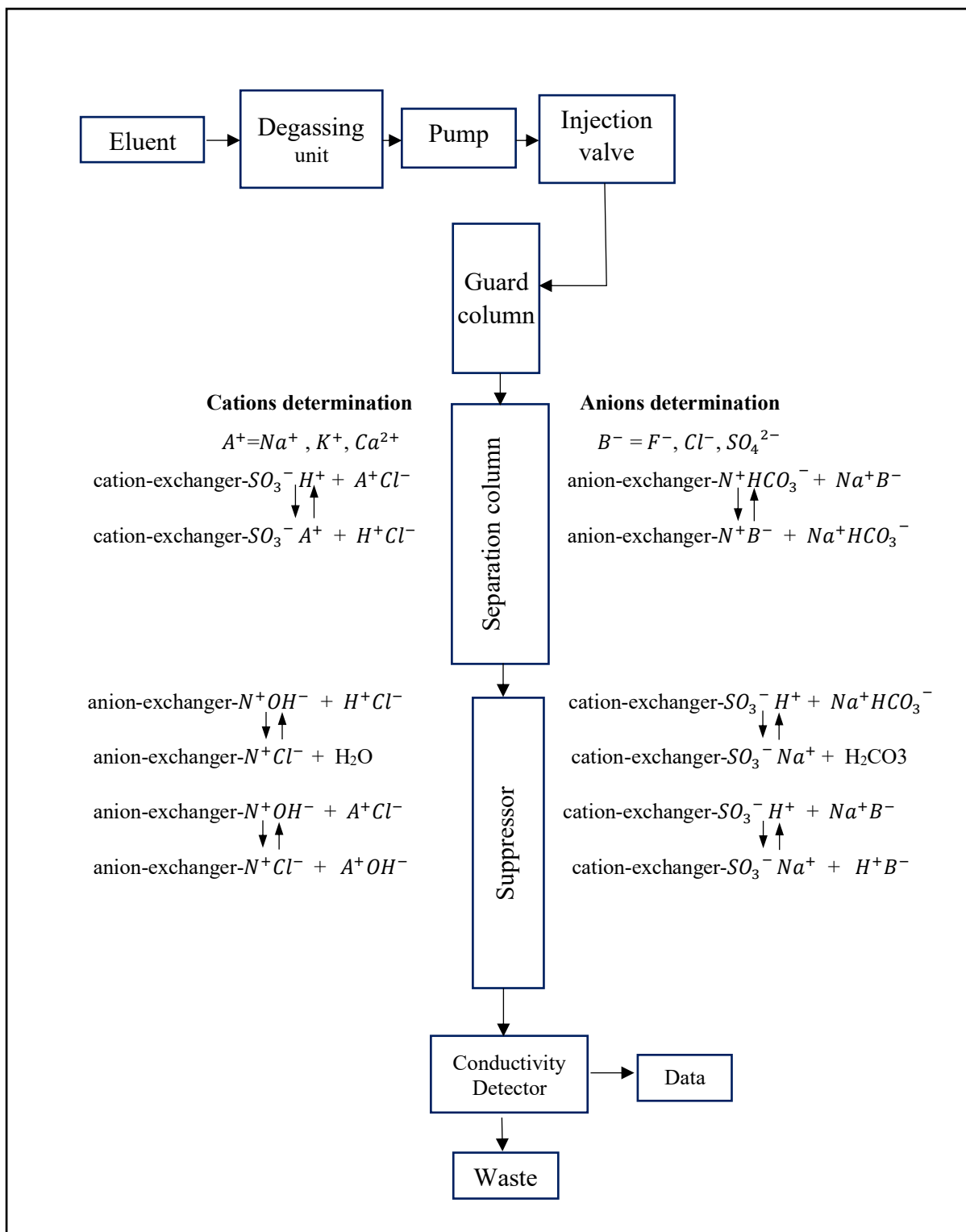


Figure 6. Schematic diagram of ion chromatography for separation of cations and anions with suppressed conductivity detection.

8 Characterization

8.1 Electrochemical impedance spectroscopy

Electrical circuits resist the flow of electrical current, which is termed electrical resistance for direct current (DC) circuits and impedance for alternating current (AC) circuits. The impedance is the ratio of potential and current for the AC system. An electrochemical impedance spectroscopy (EIS) measurement is performed by applying a small-amplitude sinusoidal excitation signal of a particular frequency to an electrochemical cell and the magnitude of the impedance and the phase shift are obtained from the measured response.

$$E_t = E_0 \sin(\omega t) \quad \text{Equation 13}$$

$$I_t = I_0 \sin(\omega t + \phi) \quad \text{Equation 14}$$

$$\omega = 2\pi f \quad \text{Equation 15}$$

where E_t and I_t are the potential and the current, respectively, at time t ; E_0 and I_0 are the amplitudes of the applied potential and the measured current, respectively; ϕ is the phase angle or phase shift between the applied potential and measured current.; ω is the angular frequency. A phase shift of 0° , 45° , and 90° is for an ideal resistor, a Warburg impedance, and an ideal capacitor, respectively.

The impedance, Z , of a system can be calculated using Ohm's law, Equation 16:

$$Z = \frac{E(t)}{I(t)} \quad \text{Equation 16}$$

The magnitude of an impedance and a phase shift of a system depends on the frequency of the signal and the system being measured.

The impedance, Z , of a system is a vector quantity that consists of a real part (Z') and an imaginary part (Z''). The impedance, Z , can be calculated using Equation 17. A plot consisting of the real part along the x-axis and the imaginary part along the y-axis is a Nyquist plot (Figure 7). The Nyquist plot was used for analyzing the recorded impedance spectra in this study.

$$Z(\omega) = (Z') - j(Z'') \quad \text{Equation 17}$$

where $j = \sqrt{-1}$, is the imaginary number.

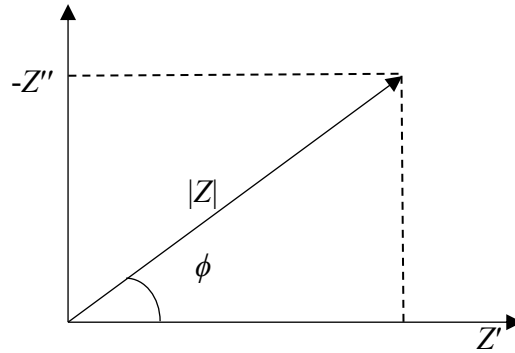


Figure 7. Nyquist plot of an impedance vector Z .

The Nyquist plot can be used for explaining the electrochemical processes in a system. This can be done by developing the best fitted equivalent circuit based on the data obtained from an EIS experiment. The equivalent circuit helps in characterizing real electrochemical processes occurring in the system. The charge transfer in a system consisting of an electrode dipped into an electrolyte solution constitutes the solution resistance, the double-layer capacitance at the electrode-solution interface, the charge-transfer resistance at the electrode surface, the diffusion of the ions to/from the electrode surface, etc. All these phenomena can be represented by a simple equivalent circuit, the so-called Randles circuit. The Randles circuit (Figure 8) consists of a resistor (due to the solution resistance, R_s) in series with the parallel combination of the double-layer capacitance, C_{dl} , and the faradaic impedance (consisting of charge-transfer resistance, R_{ct} and Warburg impedance, Z_w). The Warburg impedance is due to the diffusion of the redox species along the electrode surface.

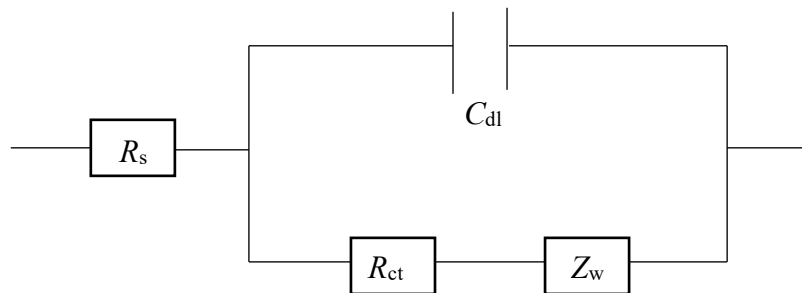


Figure 8. Randles circuit

9 Experimental section

9.1 Reagents

The ionophore, an acyclic derivative of 1,3-bis(carbazolyl)urea, used in this work had been synthesized at the University of Tartu⁶⁷. The chemicals used to prepare the membranes were all of SelectophoreTM grade. The membrane compositions comprised of high-molecular-weight PVC (Fluka), 2-nitrophenyl octyl ether (*o*-NPOE, >99%, Sigma-Aldrich), tridodecylmethylammonium chloride (TDMACl, Fluka), and tetrahydrofuran (THF, >99.5%, Sigma-Aldrich). The monomer ethylenedioxythiophene (EDOT, >97%, Sigma-Aldrich) was used for the solid contact. Sodium benzoate >99%, was obtained from Sigma-Aldrich. All other chemicals used were of analytical grade with purity $\geq 98\%$ and obtained from Acros Organics, Sigma-Aldrich, Fisher, and Fluka. Aqueous solutions were prepared with deionized water (resistivity 18.2 M Ω cm). Nitric acid (65%) from J.T. Baker was used to prepare 1 M HNO₃ for cleaning the sensors. Frozen lingonberry and cranberry were purchased from a local store in Turku, Finland.

9.2 Equipment

The reference electrode used in this study was a Metrohm AG (Herisau, Switzerland) 6.0726.100 double-junction reference electrode Ag/AgCl/3 M KCl//1 M KCl. Glassy carbon rods were used as a counter electrode and to prepare working electrodes as well. An Autolab PGSTAT30 potentiostat (EcoChemie BV, Utrecht, Netherlands) was used to perform cyclic voltammetry, electropolymerizations, and electrochemical impedance spectroscopy measurements. All potentiometric measurements (calibrations, selectivity determinations, and sample measurements) were carried out using a high input impedance (10^{15} Ω) EMF16 multichannel interfaces (Lawson Labs, Inc., Malvern, PA, USA). A 905 Titrando titrator (Metrohm AG, Herisau, Switzerland) equipped with a pair of 800 Dosino dosing systems was used for dilution during potentiometric calibrations. The pH and temperature of solutions were measured using an Orion Star A111 Benchtop pH-meter and an Orion 9157BNMD pH-electrode (Thermo Fisher Scientific, Waltham, MA, USA). A conductivity meter and a conductivity probe LE703 (Mettler Toledo, Mississauga, Canada) were used for measuring the conductivity and temperature of sample solutions. All measurements were performed at room temperature (23 ± 2 °C). An 881 Compact IC pro by Metrohm, Switzerland was used for ion

chromatography. The stationary phase used for the separation was a Metrosep anion dual 2 IC column (6.1006.100) and a Metrosep RP 2 Guard/3.5 was used as a guard column. See Table A1 for the technical specifications of the analytical column⁶⁸.

9.3 Preparation of ion-selective membranes

The membrane consisted of PVC, *o*-NPOE, ionophore, and TDMACl for the benzoate-selective electrode. THF was used as the solvent. The composition was 1.0% (dry mass) of the ionophore, 50 mol% (relative to the ionophore) of TDMACl, and a 2:1 ratio of plasticizer to PVC. The dry mass accounted for 17 wt.% of the total mass. All the dry masses were dissolved in THF. A similar membrane was also prepared without the ionophore to make the control electrodes. The membrane compositions are shown in Table 1.

Table 1. Membrane cocktail compositions of the control and the benzoate-selective membranes.

Membrane	Ionophore	Wt% (total mass)			
		TDMACl	<i>o</i> -NPOE	PVC	THF
Control	-	0.05	11.30	5.59	83
Benzoate-selective	0.17	0.05	11.19	5.65	83

9.4 Preparation of the sensors

Twelve electrodes with PVC shells consisting of six control and six benzoate-selective electrodes were prepared. The diameter of the glassy carbon (GC) disk was 3 mm corresponding to an electrode with a surface area of 0.07 cm². At first, the electrodes were polished with sandpaper (P100-P2000), diamond paste (15-1 μm), and alumina slurry (0.3 μm). Cyclic voltammetry was used to confirm that there were no traces of contamination from previous solid contacts. The electrodes were cleaned by ultrasonication in deionized water and ethanol baths, by immersion in 1 M HNO₃, and followed by ultrasonication in deionized water. For electropolymerization onto the GC electrode, a monomer solution of 0.01 M EDOT in 0.1 M KCl was prepared and mixed overnight with a stirrer for the proper dissolution of the monomer. The KCl in the monomer solution functioned both as an electrolyte and a source of the dopant, which in this case was chloride. Deaeration of the monomer solution was done by bubbling N₂ gas for at least 15 min before electropolymerization. N₂ gas was allowed to flow over the solution surface to maintain a thin layer of N₂ gas that would prevent the dissolution of oxygen into the solution throughout the electropolymerization. The galvanostatic

polymerization with a three-electrode system was performed by applying 14 μA current for 714 seconds to obtain a film with a 10 mC deposition charge with reference to the previous work of Yrjänä *et al.*⁴⁶. After electropolymerization, the electrodes were rinsed and conditioned overnight in a 10^{-2} M sodium benzoate solution. After conditioning overnight, the electrodes were rinsed and left to air-dry before drop-casting the membrane cocktails. A total of 100 μl of membrane cocktail was drop-cast in two portions of 50 μl with a waiting time of about 30 min between the two additions. The electrodes were covered with a beaker and left overnight to let the THF evaporate slowly. The dried electrodes were then conditioned in 10^{-3} M sodium benzoate solution for at least two days.

9.5 Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy measurements for all sensors were performed potentiostatically in a three-electrode system containing 0.1 M sodium benzoate solution. Before each EIS measurement, the open-circuit potential (OCP) was measured. A constant potential of 0 V vs OCP was used. A sinusoidal AC excitation signal with an amplitude of 100 mV (root mean square, RMS) was used in the frequency range of $f=100$ mHz to 100 kHz for the EIS measurement.

9.6 Potentiometry

Potentiometric measurements were performed with six electrodes simultaneously in the same solution at a time. The set of electrodes were chosen such that each set consisted of three control and three benzoate-selective electrodes. The electrodes belonging to every set of measurements were selected randomly and the set changed from day to day. Potentiometric calibrations were performed with automated serial dilution from 10^{-1} to 10^{-7} M in half-decade steps with 5 min intervals. It was practically unfeasible to deaerate the sample solution and deionized water continuously, but fresh deionized water was used before every calibration to minimize the amount of dissolved CO_2 .

Potentiometric selectivity coefficients, $K_{benzoate,j}^{pot}$ were determined for some possible interfering ions using the separate solution method⁶⁹. For interfering ions, the potentiometric measurement was done with serial dilution from 10^{-2} M to 10^{-5} M in half-decade steps with 6

min intervals. The liquid junction potentials for the primary ion i (benzoate) and the interfering ion j were calculated using the Henderson equation⁵⁴. The activity coefficients of the primary ion i and interfering ion j were calculated using the extended Debye-Hückel theory⁷⁰. The standard electrode potential, E° , for both primary ion (I^{Z_I}) and interfering ion (J^{Z_J}) was determined by extrapolating the response for the ions I^{Z_I} and J^{Z_J} to 1 M activities. The selectivity coefficient is determined by using Equation 18⁶⁹. The experimental slope obtained from the potentiometric calibration was used for calculations. The anions selected as possible interferents were malate, citrate, chloride, salicylate, tartrate, and fumarate. The interferents were selected based on the study conducted by Viljakainen *et al.*⁸ Sodium salts were used to prepare the solutions except in the case of malate. It was prepared by adding NaOH solution to a malic acid solution until the pH was near neutral.

$$\log K_{\text{benzoate},j}^{\text{pot}} = \frac{E^\circ_j - E^\circ_i}{\text{slope}} \quad \text{Equation 18}$$

where E°_j is the standard electrode potential for interfering ions, E°_i is the standard electrode potential for the primary ion (benzoate), and the slope is the experimental slope from the potentiometric calibration.

9.6.1 Sample preparation and measurement

About 100 g of frozen berries were thawed in a refrigerator for two days. The thawed berries were then mashed with a mortar and pestle. About 40 g of mashed berries were weighed in a 200 ml volumetric flask. Then 2 ml of 1 M phosphate buffer was added for ionic strength adjustment. About 50 ml of deionized water was added and the pH of the solution was adjusted to above 6.20 with 0.5 M NaOH. The volumetric flask was then filled with water. The pK_a value of benzoic acid is 4.20. The pH value was set to be above 6.20 so that the majority (ca 99%) of benzoic acid could be converted to benzoate.

The sample solution was then filtered and 50 ml of the solution was used for the measurement. The quantification of benzoate was done by following the single and double standard addition methods. Here, the single-addition method refers to the addition of 1 ml of 0.1 M sodium benzoate to 50 ml pretreated sample, whereas double-addition refers to the second addition of 1 ml of 0.1 M sodium benzoate to the sample. The potential was measured for 5 min before and after the addition of the benzoate standard solution. The conductivity of the sample was

also measured before and after the addition of the benzoate standard solution. It is most likely that the ionic strengths and the activity coefficients vary in samples with and without the addition of standard solutions. In potentiometric measurements, all the samples must have similar ionic strengths. Thus, the conductivity was measured to track the change in conductivity with the addition of standard solution and the added quantity should not result in a significant change in the conductivity.

9.7 Ion chromatography

The mobile phase used in IC was 2.0 mM NaHCO₃, 1.3 mM Na₂CO₃, and 5% acetone. The flow rate was set to 0.8 ml/min and the injection volume was set to 20 µl. Separation was done in chemical suppression mode using 0.1 M H₂SO₄ as a suppressor regenerant. The conductivity detector was used for detection. The separation was carried out at ambient temperature.

9.7.1 Sample preparation

The sample preparation was done according to the method proposed by Busra *et al*⁷¹. Some amount of thawed berries were mashed with a mortar and pestle. About 5 g of mashed berries were then weighed in a 100 ml volumetric flask. The flask was then filled with 100 ml of a solution containing methanol and water (70:30). The sample was then sonicated for 15 min. After sonication, the sample was filtered and 5 ml of the filtrate was transferred to each of five 50 ml volumetric flasks. Benzoate standard solution was added to four of the five flasks so that the concentration of added benzoate was 0.05 mM, 0.10 mM, 0.15 mM, and 0.20 mM. One sample was left without the addition of benzoate standard solution. The volume was then filled up with deionized water. The sample was then transferred to autosampler vials after filtration with a 0.45 µm pore size membrane filter.

10 Results and discussions

10.1 Galvanostatic electropolymerization

The galvanostatic polymerization was done in a three-electrode system with 0.01 M EDOT in 0.1 M KCl solution. Chronopotentiograms showed an initial fast increase in the potential to about 1.1 V that stabilized between 0.9 to 1 V. The prompt initial increase in potential can be related to the initiation of polymerization of PEDOT/Cl⁻ over the electrodes surface and the stabilization of the potential at a lower value can be related to polymerization over already formed polymer⁷². See Figure A1 for chronopotentiograms recorded during electropolymerization of control electrodes and benzoate-selective electrodes.

10.2 Electrochemical impedance spectroscopy measurements

Electrochemical impedance spectroscopy measurements were performed on control and benzoate ISEs immersed in 0.1 M sodium benzoate solution. The impedance spectra were similar to the findings presented in the study by Bobacka *et al.*⁷³. The impedance spectra (Figure 9), were typical of the impedance spectra of the solid-state ion-selective electrode. The spectra showed a semicircle in the high-frequency region followed by a diffusion line in the low-frequency region. The high-frequency semicircles correspond to the bulk resistance of the ISM in parallel with its geometric capacitance. The low-frequency line is associated with the diffusion of ions through the PEDOT and ISM layers. The geometric capacitance was calculated using Equation 19. The bulk resistance and geometric capacitances of each electrode are shown in Table 2. The developed electrodes had bulk resistances in the range of 1.8 to 3.2 MΩ and geometric capacitances in the range of 8.9 to 11.9 pF. The variation in the bulk resistance and capacitance can be due to the difference in thickness of each electrode's membrane. The absence of a large semicircle or capacitive line in the lowest frequency region shows that the PEDOT layer effectively functioned as an ion-to-electron transducer with high redox capacitance.

$$C_g = \frac{1}{2\pi f_{\max} R_b} \quad \text{Equation 19}$$

where C_g is the geometric capacitance, R_b is the bulk resistance, and f_{\max} is the frequency value that corresponds to the top of the semicircle.

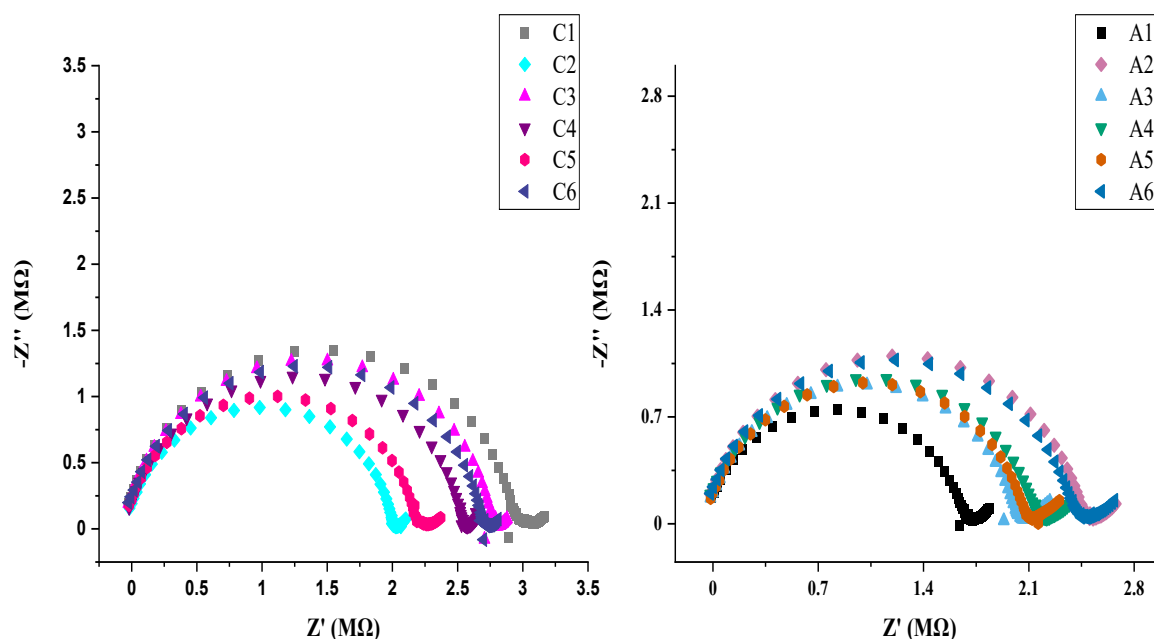


Figure 9. Nyquist plot of (a) control electrodes C1-C6, and (b) benzoate-selective electrodes A1-A6. Measurements were performed in 0.1 M sodium benzoate with the parameters $f = 100 \text{ mHz}-100 \text{ kHz}$, $E_{\text{dc}} = 0 \text{ V}$ vs. OCP, and $\Delta E_{\text{ac}} = 100 \text{ mV}$ (RMS).

Table 2. Bulk resistance (R_b) and the geometric capacitance (C_g) of the membranes on control electrodes (C1-C6) and benzoate-selective electrodes (A1-A6) with their average values and corresponding standard deviations.

	Control electrodes		Benzoate-selective electrodes		
	R_b , $M\Omega$	C_g , pF	R_b , $M\Omega$	C_g , pF	
C1	3.2	10.0	A1	1.8	10.8
C2	2.1	11.9	A2	2.7	9.4
C3	2.9	10.9	A3	2.3	11.2
C4	2.7	11.9	A4	2.4	10.5
C5	2.4	10.6	A5	2.3	10.9
C6	2.8	8.9	A6	2.3	9.4
Average	2.7	10.7	2.3	10.4	
SD	0.4	1.2	0.3	0.7	

10.3 Potentiometric measurement

10.3.1 Potentiometric calibration in pure benzoate solution

Calibrations of the electrodes were done when the electrodes were fresh and when they were two months old. The calibrations were repeated on three different days over a one-week period, in both fresh and old groups. The calibrations of the fresh and old electrodes are shown in

Figure 10. The performance of the electrodes in terms of slope, linear range, and limit of detection (LOD) was comparable to some of the ion-selective sensors reported previously^{46,74-76}. From Table A2, the variation in standard electrode potential is higher in the control group.

This could be due to some variation during drop-casting of the control membrane onto the electrode. The slopes of the fresh electrodes were Nernstian whereas the slopes of the old electrodes were near-Nernstian. The reduced slope might be due to the formation of a water layer between the solid contact and membrane or can be attributed to the leakage of ionophore from the benzoate-selective membrane to the conditioning solution upon storage. The water layer test can be performed in a non-destructive fashion but this test was not performed in this study⁷⁷. The linearity range of all electrodes remained the same. The LOD of the benzoate-selective electrodes was better than control electrodes and the LOD of the individual electrodes did not change much with time. The average response characteristics and pooled standard deviations of the electrodes, both when they were fresh and old, along with the average response characteristics and standard deviations of the electrodes obtained from the calibration in 0.01 M phosphate buffer are shown in Table 3. See Table A3 for the average response characteristics of individual electrodes, both fresh and old electrodes.

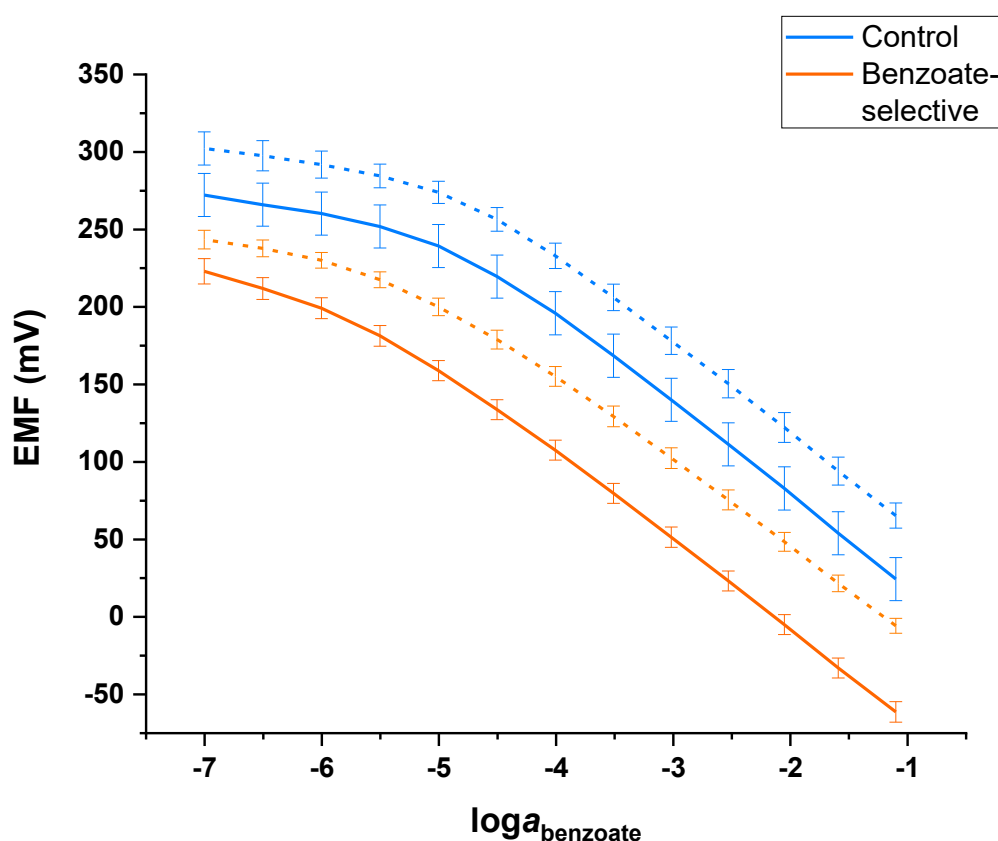


Figure 10. Potentiometric calibrations of fresh and old electrodes. The dashed lines represent two-month-old electrodes. Potentials have been adjusted by shifting the average potentials by different constant values in each group, to separate each group. The calibrations were repeated on three different days over one week period, in both fresh and old groups. The measurements from the control electrode C1 are treated as outliers in the control group. Error bars express pooled standard deviations.

10.3.2 Potentiometric calibration in 0.01 M phosphate buffer

A calibration was also performed using 0.01 M phosphate buffer as the background electrolyte to see the effect of phosphate on the response characteristics of the sensors. The calibration was carried out with automated dilution from 10^{-3} to 10^{-7} M in half-decade steps with 6 min intervals. The average response characteristics are shown in Table 3. The slope of the control electrode deteriorated in the presence of phosphate whereas the slope of the benzoate-selective electrodes remained near-Nernstian. The lower limit of linearity (LLL) was the same as in the calibrations done in a phosphate-free environment. The LOD of the benzoate-selective electrode was degraded by about 1 logarithmic unit, whereas the LOD of the control electrode did not suffer much. Consequently, careful consideration is required when choosing the phosphate buffer concentration during sample preparation. We limited the addition of the phosphate buffer to 0.01 M as the changes in the response were endurable. See Figure A2 for the calibration of electrodes in the phosphate background.

Table 3. Average response characteristics and pooled standard deviations or standard deviations of control and benzoate-selective electrodes determined from potentiometric calibrations in aqueous benzoate solution with and without 0.01 M phosphate buffer. All electrodes had the same lower limit of linearity (LLL). All linear regressions had an $R^2 \geq 0.999$. The calibrations in pure benzoate solutions were repeated on three different days over a one week period, in both fresh and old groups. The calibration in 0.01 M phosphate buffer was done one time. LOD- Limit of detection

Electrodes	Slope (mV/decade) (Avg. \pm SD)		$\log a(\text{LLL})$	$\log a(\text{LOD})$ (Avg. \pm SD)	
	Fresh	Old		Fresh	Old
Calibration in pure benzoate solution					
Control	-59.17 ± 0.21	-57.71 ± 0.72	-4.00	-5.26 ± 0.02	-5.14 ± 0.03
Benzoate-selective	-58.27 ± 0.11	-55.43 ± 0.64	-4.00	-5.89 ± 0.02	-5.71 ± 0.03
Calibration in 0.01 M phosphate buffer					
Control	-49.11 ± 0.82		-4.00	-5.21 ± 0.06	
Benzoate-selective	-56.73 ± 0.63		-4.00	-4.88 ± 0.03	

10.3.3 Potentiometric selectivity

The electrodes showed good discrimination of most of the interferents. The electrodes were selective to salicylate, which was expected as it is also an aromatic monocarboxylate with a structure similar to that of benzoate. Further, salicylic acid is also more lipophilic than benzoic acid, $\log P = 2.20$ vs. $\log P = 1.88$ in an octanol-water system⁷⁸. The other anions besides chloride are non-aromatic di- and tricarboxylates, which are less lipophilic than benzoate and salicylate, and their structure might result in the formation of less stable complexes with the ionophore, resulting in less interference. In addition, the Hofmeister series for anions shows that chloride is more lipophilic than carboxylates such as acetate. This could be the reason for the electrodes' selectivity to chloride after salicylate. The selectivity measurements were performed two times, before and after the electrodes were exposed to sample measurements. The logarithmic value of the selectivity coefficients for control electrodes and benzoate-selective electrodes before exposure to the berry samples are shown in Table 4. See Table A4 for the logarithmic value of selectivity coefficients for control electrodes and benzoate-selective electrodes after exposure to sample.

Table 4. Average potentiometric selectivity coefficients and their corresponding standard deviation determined with the separate solution method for control electrodes (C1-C6) and benzoate-selective electrodes (A1-A6) before exposure to the berry samples. The number of measurements per interfering ions was $n = 6$, all within a day, for both control and benzoate-selective electrodes.

Ion, <i>j</i>	Control electrodes		Benzoate-selective electrodes	
	$\log K_{\text{benzoate},j}^{\text{pot}}$	SD	$\log K_{\text{benzoate},j}^{\text{pot}}$	SD
Salicylate	2.27	0.13	0.34	0.12
Chloride	-0.46	0.08	-1.47	0.16
Citrate	-2.02	0.19	-2.44	0.11
Malate	-2.60	0.03	-2.62	0.13
Fumarate	-3.25	0.04	-3.53	0.05
Tartrate	-3.21	0.10	-3.73	0.09

10.3.4 Sample measurement

The concentration of benzoate was determined following the single-addition and double-addition methods. The number of measurements per electrode was five. The concentration determined with control electrodes was higher than with benzoate-selective electrodes, which is explained by the poorer selectivity of the control electrodes. The content of benzoate was higher in lingonberries than cranberries, which is also evident from the previous publication⁸. The average concentration and standard deviation determined with control electrodes and benzoate-selective electrodes are presented in Table 8 alongside results obtained with IC.

10.4 Ion chromatography method

Metrohm's ion chromatography method for the determination of benzoate was used for chromatographic separation⁷⁹. An isocratic mixture of 2.0 mM NaHCO₃, 1.3 mM Na₂CO₃, and 5% acetone was used as the eluent. The method could separate benzoate distinctly from other anions. However, a method without adding acetone was also attempted to see if acetone addition could be avoided. With this method, the separation of benzoate was not efficient in comparison with a method in which the eluent contains acetone. Without acetone, the benzoate peak was not ideally separated from malate, citrate, and tartrate. Also, with 5% acetone, the separation was completed in 45 min instead of the 60 min that were required when acetone was not used. With these outcomes, the method described by Metrohm was used for the work. Figure 11 shows the peak separation resulting from the two different methods. A mixture of standard solutions of 0.1 mM benzoate and interfering anions was prepared for the separation. The retention times of the different anions were 4.61 min for chloride, 10.13 min for benzoate, 16.82 min for malate, citrate, and tartrate, 27.31 min for fumarate, and 40.30 min for salicylate.

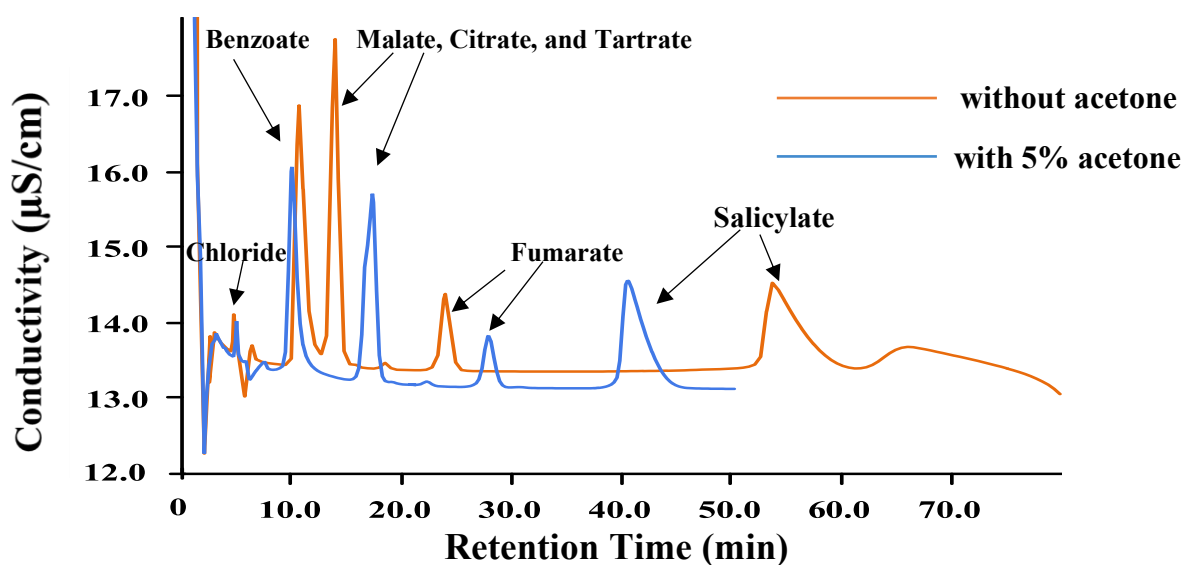


Figure 11. Comparison of peak separation of anions with and without 5% acetone in the eluent (2.0 mM NaHCO₃ and 1.3 mM Na₂CO₃). Flow rate: 0.8 ml/min.

10.4.1 Linearity

The calibration curve was plotted with peak area ($\mu\text{S/cm}$) against the concentration range of (2.5 – 100 μM). The calibration curve parameters were calculated by regression analysis. Experiments performed at five concentration levels, using six replicate injections of benzoate standard for each concentration level, gave a linear calibration curve with respect to the peak area. The calibration curve (Figure 12) was characterized by a slope of 0.0224 with a standard deviation of 0.0003, and by an intercept of -0.03 with a standard deviation of 0.02. The standard error of the regression (S) was 0.03. The correlation coefficient (R^2) value was 0.9993, which demonstrates the excellent linearity of the method.

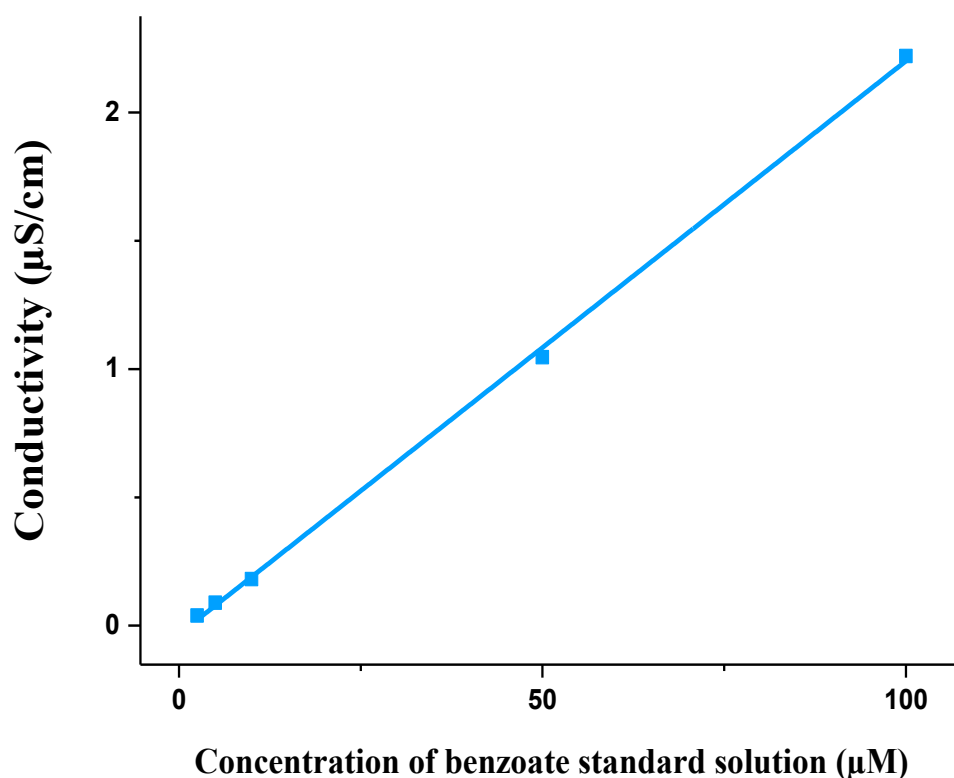


Figure 12. The calibration curve for benzoate standard concentration (2.5-100 μM). Eluent : 2.0 mM NaHCO_3 , 1.3 mM NaCO_3 and 5% acetone, flow rate: 0.8 ml/min.

10.4.2 Repeatability

The repeatability test for the retention time and peak area was performed at five different concentration levels (2.5-100 μM) of benzoate standard with six replicate injections. The relative standard deviation (RSD) ranged from 0.1% to 0.5% for retention time and from 1% to 3% for peak area. The RSD was below 5% which signifies good repeatability of measurements. The repeatability test for retention time and peak area along with their average value and RSD value at each concentration are shown in Table 5.

Table 5. Repeatability test for retention time and peak area at five different concentrations of benzoate standard solution. The test was performed with six replicate injections. Eluent : 2.0 mM NaHCO_3 , 1.3 mM NaCO_3 and 5% acetone, flow rate: 0.8 ml/min

Concentration μM	Retention Time (min)	RSD (%)	Peak Area $\mu\text{S/cm}$	RSD (%)
2.5	10.12	0.1	0.040	3
5	10.14	0.2	0.089	1
10	10.16	0.2	0.181	1
50	10.12	0.2	1.046	1
100	10.14	0.5	2.226	1

10.4.3 Standard addition method

Five samples each of cranberry and lingonberry were prepared and analyzed with IC. Analyte concentration was determined by the standard addition method. It was performed by addition of 0.05 mM, 0.10 mM, 0.15 mM, and 0.20 mM of benzoate standard to an equal amount of sample. The chromatograms of conductivity response after the addition of benzoate standard to cranberry and lingonberry samples are shown in Figure 13 and Figure 14, respectively.

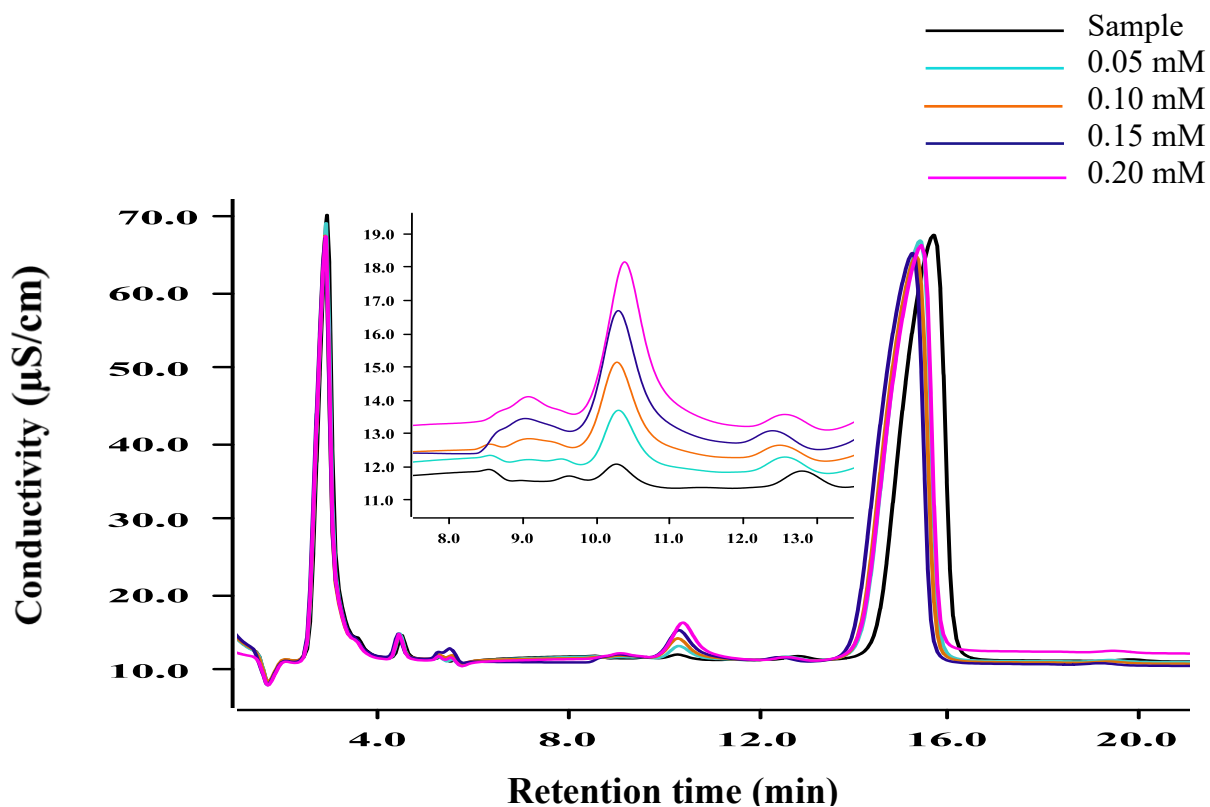


Figure 13. Chromatogram of cranberry sample showing conductivity response after standard addition of 0, 0.05, 0.10, 0.15, and 0.20 mM benzoate standard added to cranberry samples. Eluent: 2.0 mM NaHCO_3 , 1.3 mM NaCO_3 and 5% acetone, flow rate: 0.8 ml/min. The inset figure represents the magnified chromatogram of benzoate.

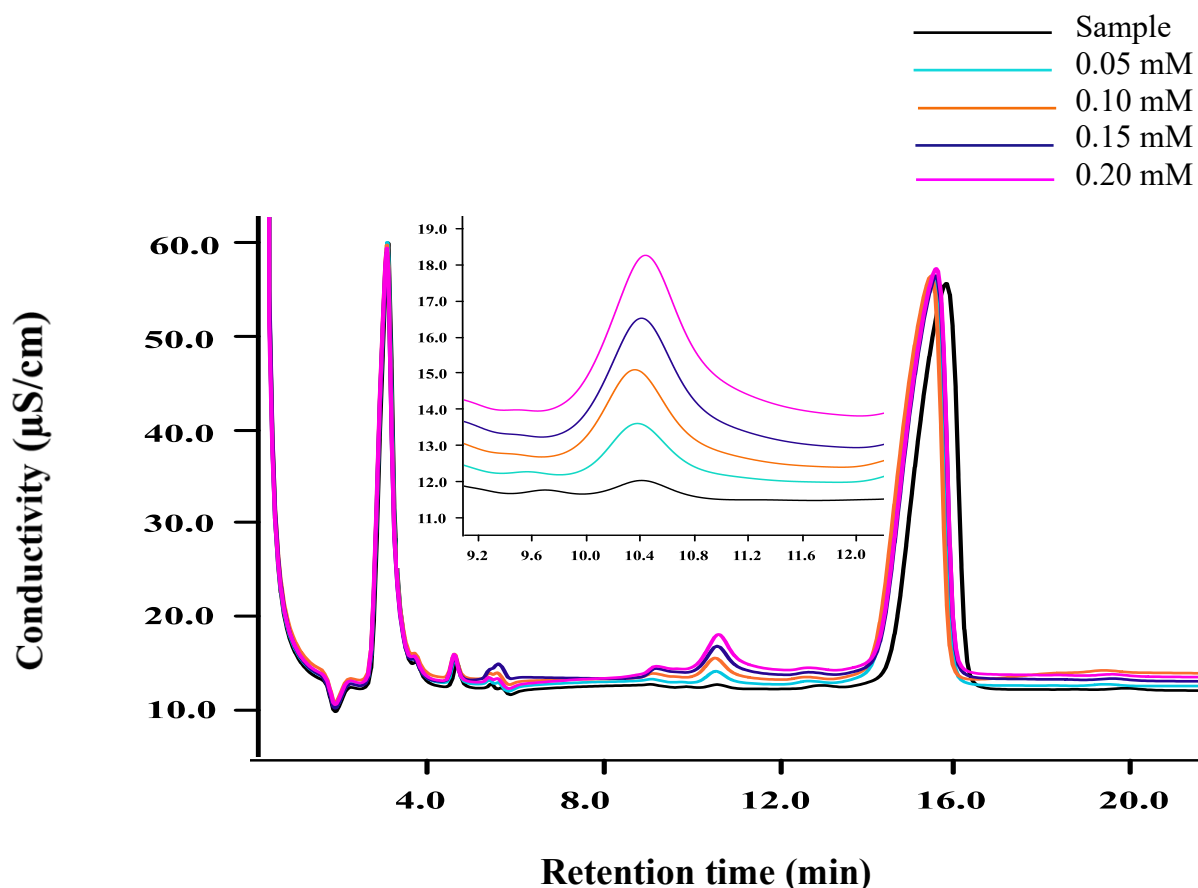


Figure 14. Chromatogram of lingonberry sample showing conductivity response after standard addition of 0, 0.05, 0.10, 0.15, and 0.20 mM benzoate. Eluent: 2.0 mM NaHCO_3 , 1.3 mM NaCO_3 and 5% acetone, flow rate: 0.8 ml/min. The inset figure represents the magnified chromatogram of benzoate.

The linear fits of the benzoate standard added to cranberry and lingonberry samples are shown in Figure 15 and Figure 16, respectively. The parameters of the calibration curve were calculated by linear regression and are presented in Table 6 and Table 7, respectively. The benzoate content was determined by taking dilution into account. The average benzoate content in cranberry and lingonberry from five replicates in each is given in Table 8 along with the result obtained with potentiometry.

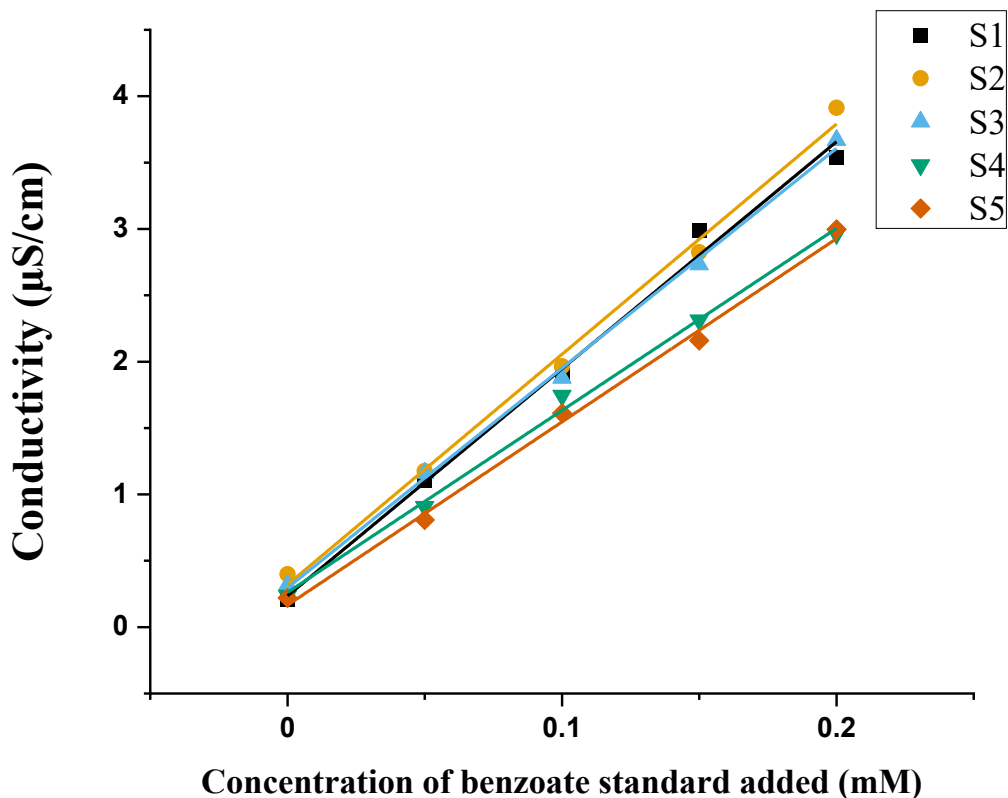


Figure 15. Calibration curves of standard addition method in IC. Addition of 0.05, 0.10, 0.15, and 0.2 mM benzoate standard solution to five cranberry samples. Eluent: 2.0 mM NaHCO₃, 1.3 mM NaCO₃ and 5% acetone, flow rate: 0.8 ml/min

Table 6. Linear fit parameters of calibration curves of five samples of cranberry. S is the standard error of the regression.

Parameter	S1	S2	S3	S4	S5
Intercept ± SD	0.24 ± 0.10	0.32 ± 0.08	0.30 ± 0.05	0.30 ± 0.06	0.17 ± 0.07
Slope ± SD	17.11 ± 0.82	17.35 ± 0.72	16.55 ± 0.42	13.71 ± 0.47	13.81 ± 0.57
S	0.13	0.11	0.07	0.07	0.09
R ²	0.9965	0.9974	0.9990	0.9983	0.9983

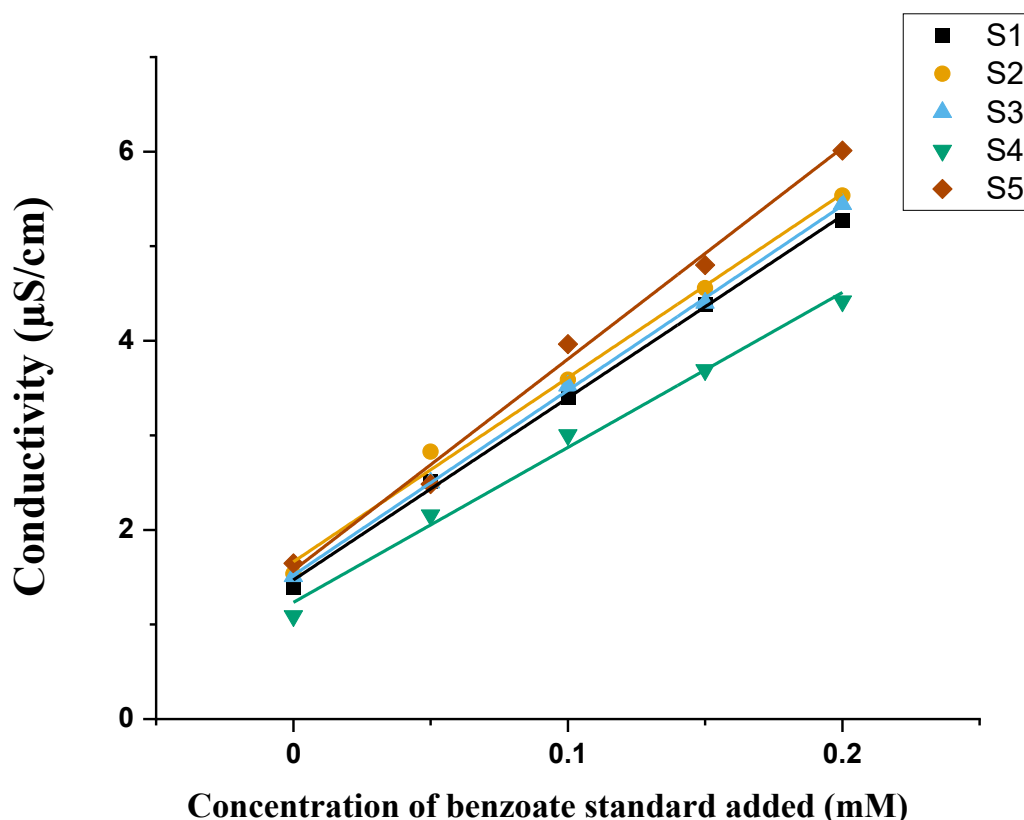


Figure 16. Calibration curves of standard addition method in IC. Addition of 0.05, 0.1, 0.15, and 0.2 mM benzoate standard solution to five lingonberry samples. Eluent : 2.0 mM NaHCO₃, 1.3 mM NaCO₃ and 5% acetone, flow rate: 0.8 ml/min.

Table 7. Linear fit parameters of calibration curve of five samples of lingonberry. S is the standard error of the regression.

Parameter	S1	S2	S3	S4	S5
Intercept ± SD	1.47 ± 0.06	1.66 ± 0.10	1.52 ± 0.03	1.23 ± 0.11	1.57 ± 0.15
Slope ± SD	19.26 ± 0.46	19.46 ± 0.85	19.54 ± 0.27	16.38 ± 0.87	22.32 ± 1.31
S	0.07	0.13	0.04	0.14	0.16
R ²	0.9983	0.9943	0.9994	0.9916	0.9934

10.5 Comparison of the result between two techniques

The benzoate content in cranberries and lingonberries was determined using potentiometry and ion chromatography. The concentration was determined potentiometrically by employing both control electrodes and benzoate-selective electrodes. Single and double standard addition methods were used. The content was determined in IC also by using the standard addition method. The amount found by potentiometry was higher than by IC. The higher concentration

resulting from the potentiometric measurement can be attributed to interference from other anions like salicylate and chloride, especially for the control electrodes. The zeta-scores were calculated with respect to the reference value determined from IC and the within-lab reproducibility standard deviations were used as standard uncertainty estimates. It is expected that these within-lab reproducibility standard deviations are relatively smaller than the combined standard uncertainties. Therefore, the use of within-lab reproducibility standard deviations as the estimate for standard uncertainty would artificially increase our zeta-score values making our test more stringent. The zeta-scores of benzoate-selective electrodes were $|zeta| \leq 2$, that shows the performance of the benzoate-selective electrodes to be satisfactory, which further implies that the benzoate content determined by the benzoate-selective electrodes was comparable to IC. Hence, the fabricated benzoate-selective electrodes were usable for benzoate determination in berries. Average concentration and standard deviation determined by potentiometry and IC have been summed up in Table 8.

Table 8. Average concentration with corresponding standard deviation of benzoate in cranberry and lingonberry samples, determined by potentiometry and IC. The standard deviations were pooled standard deviations in the case of potentiometric measurements. The zeta-scores were calculated with respect to the reference value determined from IC. The number of sample replicates was $n = 5$, measured over five different days, one sample per day, in both potentiometric and IC measurements.

Berry	Control electrode		Benzoate-selective electrode		IC
	Single-addition	Double-addition	Single-addition	Double-addition	
Cranberry					
Average (g/kg)	0.49	0.92	0.28	0.23	0.17
SD	0.05	0.07	0.05	0.04	0.03
Zeta-scores	5.5	9.8	1.9	1.2	
Lingonberry					
Average (g/kg)	1.32	1.50	1.12	1.08	0.88
SD	0.18	0.13	0.10	0.20	0.07
Zeta-scores	2.3	4.2	2.0	0.9	

11 Conclusion

PVC membrane-based benzoate-selective solid-contact electrodes were fabricated. Control electrodes, which had a similar membrane but no ionophore, were also prepared. The electrodes were characterized with EIS. The impedance spectra of electrodes were characteristic of that of PVC membrane-based solid-contact ion-selective electrodes. The PEDOT used as a solid contact could effectively function as an ion-to-electron transducer between the ISM and the electronically conducting substrate. The calibration of the electrodes showed that the slopes were Nernstian when they were fresh and near-Nernstian when they were two months older. However, the overall electrodes' performance in terms of linearity, slope, and limit of detection had not deteriorated much over the course of two months, which shows the electrodes to be fairly robust and stable. The electrodes discriminated most of the other interfering anions tested, whereas moderate selectivity towards a more lipophilic structural analogue, salicylate was also observed. The electrodes could be conveniently used for the determination of benzoate in the berry samples. Ion chromatography was also used as a method of analysis for the determination of benzoate concentration. In both analytical methods, the standard addition method was employed for the determination of benzoate in the sample to minimize the matrix effect. Comparison of both methods showed that the concentration determined by potentiometry was slightly higher but roughly similar to IC. Hence, the developed electrodes could be reliably used for benzoate determination in the berry samples. The scope of application could be broadened to encompass the determination of benzoate concentration in other food products, beverages, pharmaceuticals, and cosmetics. This could be a faster, easier, simpler, and more economical alternative to other analytical methods for the routine quantification of benzoate.

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Appendix A

Appendix A.1 Technical specifications of the analytical column

Table A1. Technical specifications of the analytical column

Substrate	Polymethacrylate with quaternary ammonium groups
Column dimensions	75 * 4.6 mm
Column body	Stainless steel
Standard flow	0.8 ml/min
Maximum flow	1.2 ml/min
Maximum pressure	7 MPa
Particle size	6 µm
Organic modifier	5%
pH range	1-12
Capacity	17 µmol(Cl ⁻)

Appendix A.2 Galvanostatic electropolymerization

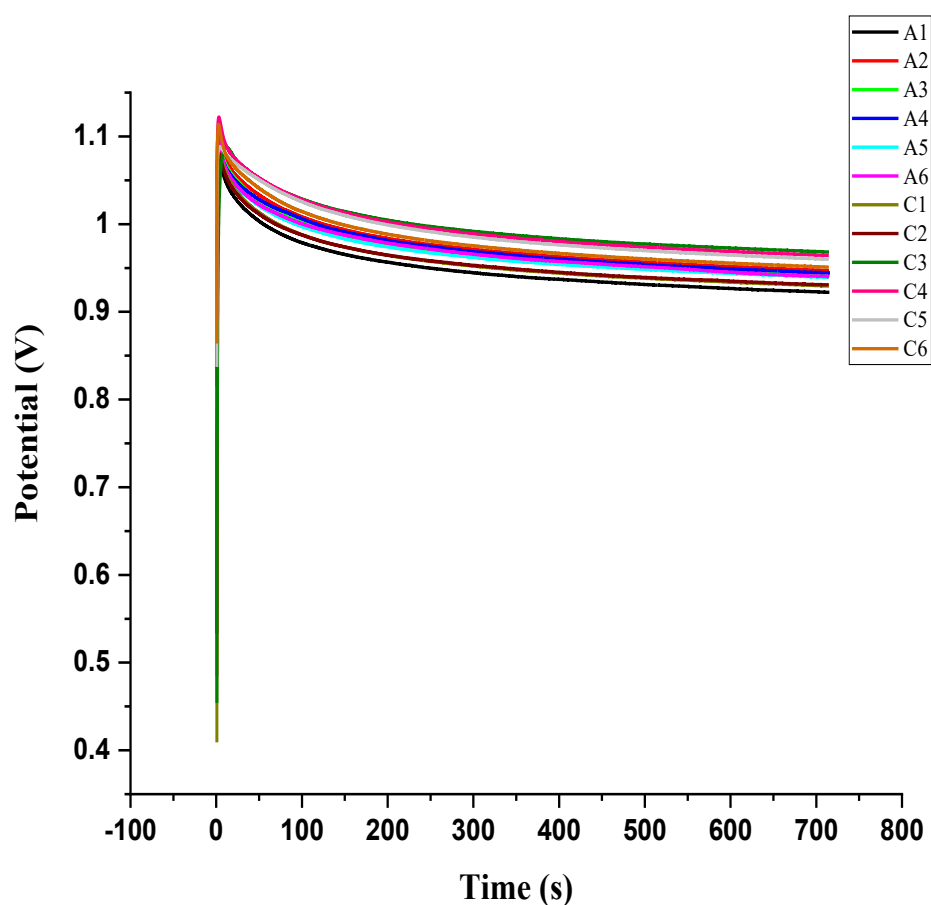


Figure A1. Chronopotentiograms of control electrodes (C1-C6) and benzoate-selective electrodes (A1-A6), recorded during galvanostatic electropolymerization in a three-electrode system containing 0.01 M PEDOT in 0.1 M KCl.

Appendix A.3 Potentiometric calibrations

Appendix A.3.1 Potentiometric calibration in aqueous benzoate solution

Table A2. Average standard electrode potential (E°) and pooled standard deviation of electrodes in the fresh and old groups. The number of calibrations done was three over a one week period, in both fresh and old groups.

Electrodes	Fresh		Old	
	Average (mV)	SD	Average(mV)	SD
Control	-9	36	-20	18
Benzoate-selective	-50	11	-35	6

Table A3. Average response characteristics of individual control (C1-C6) and benzoate-selective electrodes (A1-A6), fresh and old, determined from potentiometric calibrations. All electrodes had the same lower limit of linearity (LLL). All linear regressions had an $R^2 \geq 0.999$. The number of calibrations done was three, over a one week period, in both fresh and old groups. LOD- Limit of detection

Electrodes	Slope (mV/decade)		$\log a(\text{LLL})$	$\log a(\text{LOD})$	
	Fresh	Old		Fresh	Old
C1	-59.07	-58.00	-4.00	-5.27	-5.08
C2	-59.10	-58.50		-5.24	-5.14
C3	-59.38	-57.57		-5.25	-5.18
C4	-59.48	-57.20		-5.28	-5.16
C5	-59.08	-57.59		-5.29	-5.15
C6	-58.90	-57.35		-5.24	-5.12
A1	-57.93	-55.13		-5.87	-5.68
A2	-58.71	-55.66		-5.87	-5.67
A3	-58.09	-55.15		-5.91	-5.72
A4	-57.89	-55.91		-5.92	-5.75
A5	-58.25	-55.24		-5.86	-5.66
A6	-58.80	-55.08		-5.88	-5.75

Appendix A.3.2 Potentiometric calibration in 0.01 M phosphate buffer

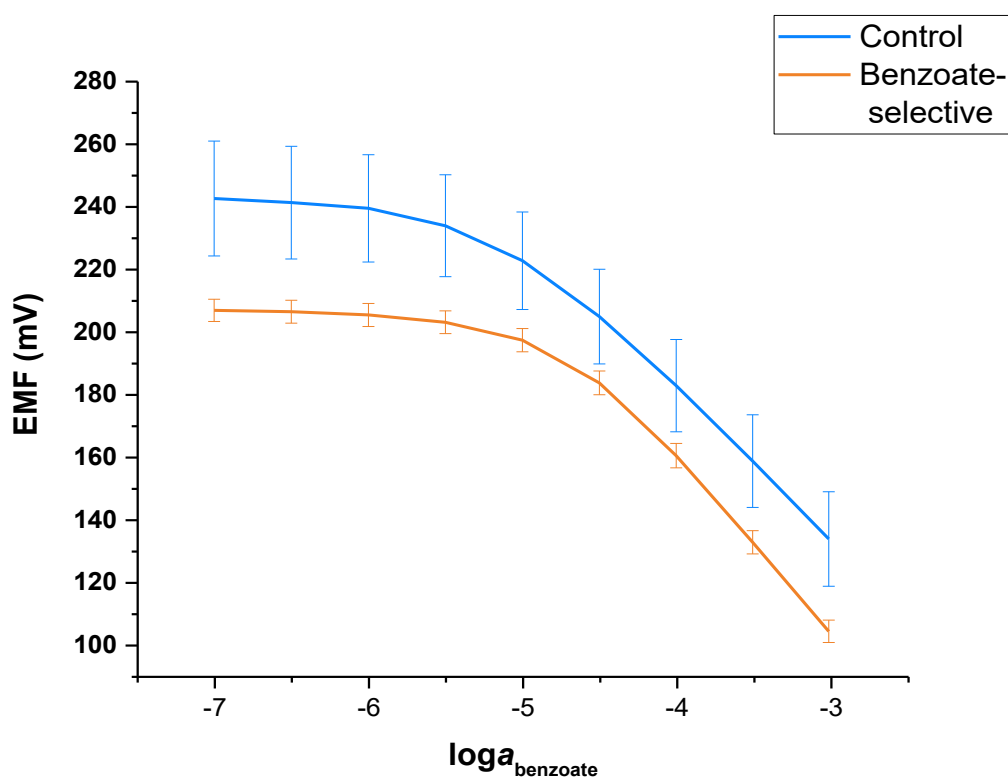


Figure A2. The potentiometric calibration of control electrodes and benzoate-selective electrodes in 0.01 M phosphate buffer. Error bars express the standard deviations. The measurements from control electrode C1 have been treated as the outliers.

Appendix A.4 Potentiometric selectivity after exposure to berry samples

Table A4. Average potentiometric selectivity coefficients and their corresponding standard deviation determined with the separate solution method for control electrodes (C1-C6) and benzoate-selective electrodes (A1-A6) after exposure to the berry samples. The number of measurements per interfering ions was $n = 6$, all within a day, for both control and benzoate-selective electrodes.

Ion, j	Control electrodes		Benzoate-selective electrodes	
	$\log K_{\text{benzoate},j}^{\text{pot}}$	SD	$\log K_{\text{benzoate},j}^{\text{pot}}$	SD
Salicylate	3.33	0.10	0.57	0.02
Chloride	-0.43	0.03	-1.84	0.04
Malate	-2.43	0.10	-2.57	0.07
Citrate	-2.87	0.09	-2.50	0.04
Fumarate	-3.25	0.04	-3.53	0.05
Tartrate	-3.28	0.08	-3.79	0.03