Determination of acetate in wine: comparison between ion chromatography and ion-selective electrodes

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Abstract

This thesis was carried out in the Laboratory of Analytical Chemistry at Åbo Akademi University in collaboration with the Analytical Chemistry Laboratory at Tartu University. This work was done under the supervision of Rose-Marie Latonen, Johan Bobacka, and Ivo Leito.

In this thesis, potentiometric solid-contact ion-selective electrodes (SC-ISEs) selective to acetate were fabricated and used to determine acetate concentration in wine. The potentiometric determination of acetate concentration in wine was compared with measurements made by conventional ion chromatography (IC). The solid-contact (SC) acetate-selective electrodes were fabricated by galvanostatic electropolymerization of poly(3,4-ethylenedioxythiophene) (PEDOT) on the surface of glassy carbon electrodes as the SC layer and drop-casting the acetate-selective ionophore 1,3-bis(carbazolyl)urea derivative containing membrane over the PEDOT layer. Both quantitative and qualitative determination of acetate in wine by SC-ISEs was evaluated by using the standard addition method. In parallel, the analysis conditions for acetate determination with ion chromatography were optimized to obtain better separation between lactate, acetate and formate by varying parameters such as the flow rate and the concentration of eluent components. The concentration of acetate in the same wine sample was measured by IC and potentiometric ISE techniques. A significant concentration difference was obtained between the two techniques. A higher concentration of acetate was obtained by the SC-ISEs compared to IC. It can be explained by the presence of lactate and formate ions in the wine sample that contribute to the ion activity measured by the acetate-SC-ISEs. Gradual leakage of the ionophore from the ISE membrane may also contribute to insufficient selectivity of the acetate-SC-ISEs used in this work.

Key words: acetate, ion chromatography, solid-contact ion-selective electrodes

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Abbreviation list

SC	Solid contact
ISEs	Ion-selective electrodes
IC	Ion chromatography
VA	Volatile acidity
RPLC	Reversed-phase liquid chromatography
HPLC	High-performance liquid chromatography
GC	Gas chromatography
PEDOT	Poly(3,4-ethylenedioxythiophene)
ISM	Ion-selective membrane
CWE	Coated-wire electrode
PVC	Polyvinylchloride
ECP	Electrically conducting polymer
CPs	Conducting polymers/conjugated polymers
PA	Polyacetylene
PPy	Polypyrrole
PTh	Polythiophene
PANI	Polyaniline
CV	Cyclic voltammetry
EIS	Electrochemical impedance spectroscopy
EMF	Electromotive force
EDOT	3,4-ethylenedioxythiophene
GC	Glassy carbon
SD	Standard deviation
RSD	Relative standard deviation
SSM	Separate solution method

1. Introduction

Volatile acidity (VA) of wine, the content of low molecular weight fatty acids in wine, is characteristically regarded as an important indicator of spoilage of wine having an odor of vinegar. The total acidity of wine results from the presence of VA and non-volatile or fixed acids. Tartaric, malic and citric acids are three main acids in wine grapes while acetic and succinic acids are the key components formed during wine manufacturing. Most concerns of wine manufactures are put on acetic acid as it contributes to over 93 % of volatile acidity in wine whereas the rest is from the contribution of carbonic, sulfurous, lactic, formic, butyric and propionic acids [1]. Acetic acid is a common indicator for routine analysis in many wine laboratories. In Hanekom's study, acetic acid content measured in 25 wines was varying from 293.98 mg/L to 747.05 mg/L [2]. Ethyl acetate is perceived as the key indicator in wine spoilage as it has the odor of "nail-polish remover" which can significantly affect the sensory perception of VA with off-flavor [3]. There are two potential processes, which can cause wine spoilage. At the beginning of wine fermentation, formation of large amounts of acetic acid might cause it. This formation of acetic acid is caused by ester taint, which is correlated to presence of microorganism species (Pichia anomala, Kloeckera apiculata, and Hanseniaspora uvarum). Ester taint also contributes to the existence of acetate ester particularly ethyl acetate which dominates the off-flavor in wine. Another potential spoilage process of wine comes when the wine is stored in oak barrels, from the growth of acetic acid bacteria, yielding small amounts of acetic acid and ethyl acetate; also, lactic acid bacteria may attribute to significant amounts of acetic acid besides lactic acid [1].

The legally permitted (maximum) acetic acid content for wine quality control in the United States is set at 1.2 g/L for white wine and 1.4 g/L for red wine, and higher acetic acid content in wine than this limit is regarded as objectionable or spoiled. The permissible limitation of ethyl acetate content is set to 12.3 mg/L while the level in defective wines can rise to 0.15-0.2 g/L with off-flavor of "nail polish remover" [1]. Thus, the determination of acetate content is crucial for the quality control of wine. Many available quantitative techniques such as ion suppression reversed-phase liquid chromatography (RPLC), high-performance liquid chromatography (HPLC), non-suppressed/suppressed ion chromatography (IC), gas chromatography (GC), and capillary isotachophoresis techniques have been used for determination of acetate content [3].

IC, providing the advantages of efficiency and easy-to-use, has been employed for analysis and separation of various ionic solutes. IC is regarded as a crucial tool in modern ion analysis in which both quantitative and qualitative analysis of anions or cations can be achieved simultaneously during the separation process, with characteristic retention times of ions [4].

Meanwhile, a simple quantitative analysis method for wine quality control from the wine making process to its long-time storage is desirable. The aim of this thesis is to study the suitability of a potentiometric solid-contact acetate-selective electrode, a handy, rapid and inexpensive device, for qualification and quantification purposes. The solid-contact acetate-selective electrode is evaluated for monitoring the acetate concentration in wine, in comparison with the conventional ion-exchange chromatography technique.

Furthermore, the aim is to optimize the analysis conditions to obtain better separation performance between lactate, acetate and formate by anion-exchange IC as they typically have similar affinities for the stationary phase thus resulting in similar retention times. In parallel, the aim is to study the potentiometric performance of the acetate-selective electrode in real wine samples and to determine the effect of possible interfering ions such as lactate and formate to the measurement result.

2. Theory

2.1 Solid-contact ion-selective electrodes

Ion-selective electrodes (ISEs) are a subgroup of electrochemical sensors which selectively respond to the activity of a target ion in sample solution and the chemical information (ion activity) is converted to an electrical signal (potential), which is analyzed in real time for further processing or recording [5]. Easy-to-use, compact size, minimal power consumption and low manufacturing costs have drawn attention to the use of ISEs to determine various inorganic and organic ions in the fields of medicine, environmental monitoring and food quality and process industry control [6].

ISEs can be made of glass, inorganic crystals, and polymeric membranes. The first ISE application, i.e. the glass pH electrode, was invented by Haber and Klemensienwicz at the beginning of the 20th century. The working principle is based on measurement of the relation between the potential and the pH of a glass membrane studied by Cremer. However, after 1936, the commercialization of glass pH electrodes and the pH meters by Beckman made these electrodes applicable to a wider use. Almost at the same time the theory behind the response of a glass electrode, the derivation of the Nikolsky equation and the invention of the term "selectivity constant" were published in Nikolsky's studies. Although the ISE with a glass membrane has the longest history, it has limited applications only in determination of H^+, Na^+, K^+, Li^+ and Ag^+. The need for more versatile applications promoted the development of other types of ISEs. Though introduced over 90 years ago, ISEs with crystalline membranes drew much attention in 1966 which was regarded as the breakthrough of crystalline-based ISEs with extremely high selectivity to fluoride and with less interference compared to that of the pH glass electrodes. ISEs with polymeric membranes especially containing neutral or charged ionophores (ionophore-based ISEs) are the largest group of ISEs nowadays [7].

The ISEs with polymeric membranes incorporate neutral or charged species (ionophores) which selectively bind the target ions. Thus, the selectivity and the detection limit of ISEs are dependent on the composition and components of the ion-selective membrane (ISM). The schematic diagram of a conventional ISE is presented in Figure 1, left. In a conventional polymeric membrane-based ISE, an internal solution forms a liquid contact between the ion-selective membrane and the internal reference electrode. Ionic activities in the sample solution are detected by the ion-selective membrane and analyzed as the potential difference between the internal reference electrode and the external reference electrode with a high input impedance potentiometer [7].

Although, conventional ISEs have excellent stability and reproducibility, it is desirable to substitute the internal filling solution by a solid material for improved portability and less maintenance while retaining the analytical characteristics of ISE. Many efforts have been made to substitute the internal filling solution, to minimize maintenance, to prevent evaporation and to eliminate effects from surrounding pressure and temperature during usage and storage. The introduction of the coated-wire electrode (CWE) in early 1970s was the base for further development of solid-contact (SC) ISEs. The CWE was based on a simple design

where the ion-selective membrane was directly coated on the conductive substrate. However, poor potential stability of the CWE was critical and linked to charge transfer blockage and ill-defined potential occurring between the ionically conducting ISM and the electronically conducting electrode substrate [5]. Moreover, the internal solution volume in the conventional ISEs hinders their miniaturization, especially in clinical and biological applications [7]. Therefore, solid-contact ISEs with improved potential stability have later been developed.

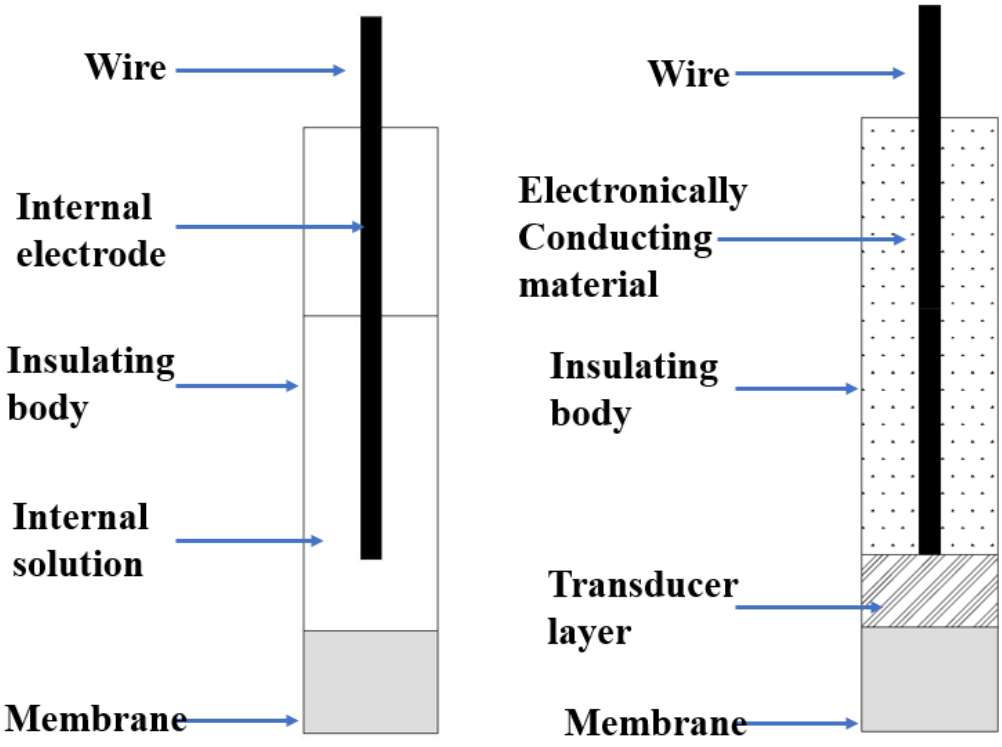

Figure 1. Schematic diagrams of a conventional ISE (*left*) and a typical solid-contact ISE (*right*).

Introduction of an intermediate solid-contact (SC) layer (Figure 1, right) of an electroactive material, deposited on the surface of the electrode substrate, resolved the charge transfer problem which led to the preliminary development of SC-ISEs. Electroactive materials with high redox capacitance or high double layer capacitance are often used as SC layers in ISEs. Electrically conducting polymers (ECPs), one type of electroactive materials, possess mixed conductivity, i.e. electronically and ionically, resulting from the redox capacity obtained during their redox reaction [8]. ECPs efficiently work as ion-to-electron transducers between the ionically conducting ISM and electronically conducting electrode substrate. The

developed SC-ISEs have the advantages of improved robustness, possibility of miniaturization, minimal maintenance and easy mass production of potentiometric ion sensors [7]. A typical polymeric membrane of a SC-ISE is composed of polyvinylchloride (PVC), a plasticizer, a lipophilic salt and an ionophore selective to the target ion. These components are dissolved in an organic solvent such as tetrahydrofuran and the cocktail thus formed is deposited onto the surface of an ECP-based electrode [9]. After evaporation of the solvent, an ion-selective membrane is formed.

2.1.1 Ionophores

Ionophores, used as the ion recognition sites providing selectivity, are immobilized in the plasticized PVC matrix of the ion-selective membrane and this membrane is then coated on the ECP film in ECP-based ISEs to make a potentiometric chemical sensor. Ionophores are organic lipophilic substances with selective binding abilities to specific ionic and neutral species [7]. Both neutral and charged ionophores are available commercially to make chemical sensors to be used in routine analysis, such as clinical analysis [10]. Over 70 analytes including inorganic ions and organic ions, and even some non-ionic species have been quantified by ionophore-based ISEs [11]. Ionophores are the key determining components for the ISE's performance, selectivity and detection limit. 1,3-bis(carbazolyl)urea derivative (shown in Figure 2) is one of 22 acyclic synthetic receptors, which have different numbers and geometric arrangements of hydrogen-bond donors and hydrophobic moieties. It is selected as an ionophore in acetate-selective ISE used in this study due to its strong binding ability to 5 (acetate being one of them) out of 11 monocarboxylates, as studied by Martin et al. [12].

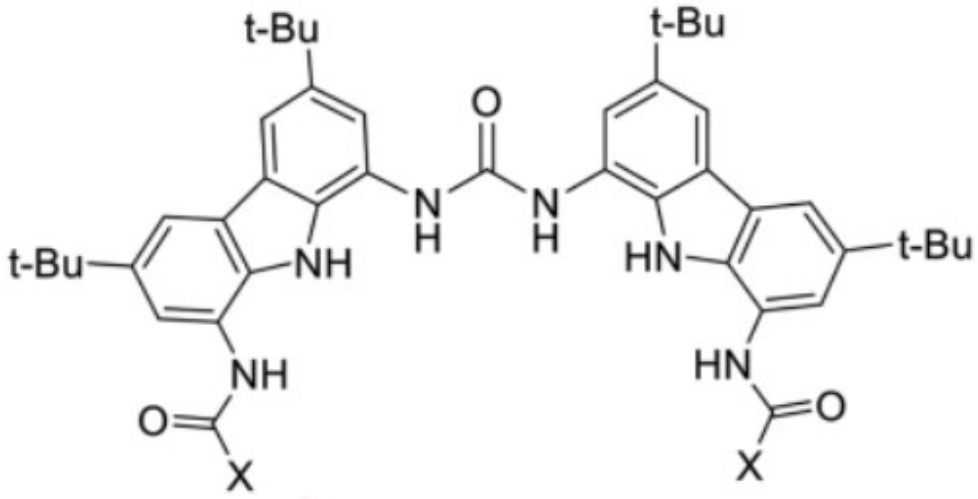

Figure 2. Chemical structure of 1,3-bis(carbazolyl)urea derivative ionophore, X= *1-amino naphthyl.*

2.2 Electrically conducting polymers

ECPs are composed of chains of carbon atoms connected by alternating single and double bonds forming a π-conjugated system and they are also called as conjugated polymers (CPs). ECPs can be obtained by chemical or electrochemical redox reactions during which the polymer is formed in a conductive state [13]. ECPs are insulators or semiconductors in their neutral state. Their conductivities can be substantially increased by removing electrons (oxidation/p-type doping) from or adding electrons (reduction/n-type doping) to the conjugated polymer backbone [14].

An oxidant is applied for chemical synthesis of ECPs in solutions, while electropolymerization is a common way to deposit ECPs on the surface of conducting substrates [15]. The simplicity and reproducibility of electrochemical polymerization make it preferred. One of the main advantages of electropolymerization is the easily-controlled reaction rate by the potential or current density applied to the electrode. Another advantage is that the thickness of well-adhering films can be controlled by the integrated charge used for electrosynthesis. For electrochemical synthesis of ECPs, potentiodynamic (cyclic voltammetry), galvanostatic (chronopotentiometric), or potentiostatic (chronoamperometric) electropolymerization methods can be used [16].

A one-compartment three-electrode electrochemical cell is often employed in electrochemical polymerization. A working electrode, a counter electrode, and a reference electrode are three parts of this cell. The surface area of the counter electrode should be larger than that of the working electrode in order to reduce the polarization of the counter electrode. The working electrodes are made of metals (e.g. Pt, Au, Ni), alloys (e.g. stainless steel), glassy carbon (GC) or conductive oxides (e.g. indium tin oxide coated glass electrode) [13].

The ECPs have been used for modification of conventional electrodes for over 30 years [10]. The improved electrocatalytic properties of modified electrodes and decreased redox potentials occurring at the electrode surface make ECPs possible to be used in diversified applications. Chemical information such as concentration, activity, partial pressure can be transduced by ECPs into electrical, electrochemical or optical signals [10]. Therefore, conjugated polymers (CPs) are useful as transducers in chemical sensors. Polyacetylene (PA), polypyrrole (PPy), polythiophene (PTh), polyaniline (PANI) and their derivatives are the most commonly used ECPs [17].

Poly(3,4-ethylenedioxythiophene) (PEDOT), one derivative of PTh, is mostly used in applications such as electrochromic devices, biosensors, solid-state ion sensors and its molecular structure is shown in Figure 3. When it is oxidized by p-type doping, it stands out with high conductivity without compromising its advantageous properties such as thermal and chemical stability and swift electrochemical switching [18, 19].

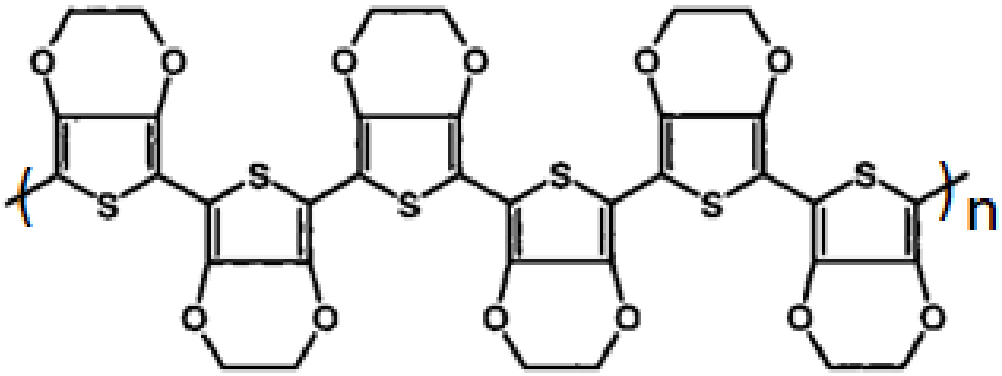

Figure 3. The chemical structure of conducting polymer - poly(3,4-ethylenedioxythiophene).

2.2.1 Galvanostatic electropolymerization method

Galvanostatic electropolymerization is a one-step and beneficial method to deposit ECPs on the surface of an electrode substrate with well-controlled synthesis efficiency. It stands out among the other methods of electropolymerization of ECPs due to its simplicity and no further change in polymer structure after the polymer is formed in its doped state [20]. A constant current with optimized current density is applied on a predetermined surface of the working electrode for galvanostatic electropolymerization in the one-compartment three-electrode electrochemical cell. The electropolymerization of the ECP with a required thickness needs a specific reaction time. The longer reaction time signifies a thicker ECP layer. The polymerization reaction proceeds at a constant rate. The potential between the working and reference electrodes can be recorded for further analysis. The charge consumed during the electropolymerization can be calculated as a function of time [21, 22].

2.3 Characterization methods

2.3.1 Cyclic voltammetry

Cyclic voltammetry (CV) is a reversible electrochemical technique which can be used both for potentiodynamic electropolymerization of ECPs and characterization of the redox behavior of synthesized polymer films. CV is mostly used to study unknown electrochemical systems and reactions occurring on the surface of the working electrode. In CV a potential is applied on the working electrode which is changed linearly over time and the direction of the potential ramp is switched after a given time interval to make a potential scan in a triangle waveform against a reference electrode in a three-electrode electrochemical cell. This electrochemical cell is made of a working electrode, a counter electrode, and a reference electrode. The current between the working electrode and the counter electrode is recorded. By plotting the recorded current versus the applied potential on the working electrode, a cyclic voltammogram is obtained [23].

During electropolymerization process in an electrolyte solution containing the monomer, the ECP film deposited on the surface of the working electrode keeps growing due to coupling of monomeric and dimeric radical species as well as due to transfer of electrolyte and solvent in

and out from the growing film. During characterization of the redox behavior of the ECP film in a monomer-free electrolyte solution, the polymer deposited on the working electrode alternatively undergo reduction and oxidation reactions, thus changing its state from neutral to conducting, as the potential is swept back and forth. A current-potential curve is recorded during this process and the shape of the curve, related to the electrolyte and solvent, the scan rate of applied potential, and thickness of the deposited film, gives information about the reversibility of the electrode reaction, charging capacity, charge transfer processes etc.[24, 25].

2.3.2 Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) can provide information about mass transfer, ion diffusion, reaction kinetics and mechanisms of electrochemical processes and capacitance of ECP-modified electrodes [26]. For example, EIS has been extensively used to study PEDOT and PTh films in aqueous solutions [27, 28]. Compared to other electrochemical methods, EIS has advantages in studying systems at equilibrium as well as processes at different reaction rates. Recorded data can be analyzed using equivalent circuits. An equivalent circuit is a theoretical circuit consisting of all the electrical elements (resistance, capacitance and inductance) arranged in simple fashion to represent the electrical characteristics of the system under observation. There are many possibilities to rearrange two or more circuit elements to yield the same impedance; therefore, many equivalent circuits can be used to describe a certain system. However, the one representing the closest fit with the data will be used.

Recorded impedance data can be studied using the Bode diagram and the Nyquist diagram. A Bode diagram is a plot of the phase angle (in degrees) versus frequency or the magnitude of impedance versus frequency. The Bode plot is used for analyzing frequency response of a system. The Nyquist diagram is a plot of the imaginary part of impedance (Z”) versus the real part (Z’) as the frequency is varied usually from 1 mHz to 1 MHz [24]. The Nyquist diagram was used for analyzing recorded impedance data in this study.

2.4 Standard addition methods for potentiometric measurements with ISEs

Single-addition and double-addition methods are standard addition methods which can be used in potentiometric measurements with ISEs. Resolving the tricky situation, without knowing activity coefficients and partial complexation of the analyte, standard addition method is considered as a useful tool particularly in potentiometric measurements with ISEs. When the single-addition method is used, it is assumed that a sample with unknown concentration of analyte is to be analyzed. The activity coefficient (γ) of the analyte is not known; additionally, only a fraction (β) of target analyte exists as free ions. Thus, the free ion activity (a) of the target ion in the sample can be given as Equation 1:

$$a=C^{ionized}\gamma=C^{total}\gamma\beta \qquad \textit{Equation 1}$$

The electromotive force (EMF) value corresponding to this activity is given by Equation 2.

$$E_1 = E^0 + S\log a^{unknown}=E^0 + S\log C^{total} + S\log\gamma + S\log\beta \qquad \textit{Equation 2}$$

After addition of a fixed amount (ΔC^{total}) of known concentration of target ion in the analyte solution, the EMF will change and is given by Equation 3:

$$E_2 = E^0 + S\log a^{processed}= E^0 + S\log (C^{total} +\Delta C^{total}) + S\log\gamma + S\log\beta \qquad \textit{Equation 3}$$

The total concentration of the target analyte is then given by

$$C^{total}=\frac{\Delta C^{total}}{10^{\frac{E2-E1}{S}}-1} \qquad \textit{Equation 4}$$

While using Equation 4, the values of γ and β are not considered for calculation and the total concentration of analyte in native sample can be obtained [29].

When we use the double-addition method, it is assumed that we have no idea whether the calibration parameters behave in the same way both in an unknown sample and in standard solutions. $C^{ionized}=C^{total}=C$ is assumed when applying this method.

The native sample EMF is given as

$$E_1 = E^0+ S\log\gamma + S\log C \qquad \textit{Equation 5}$$

where E^0, S might not behave the same both in simple standards and in the sample. ΔC of the analyte is added to the native sample and the corresponding EMF is

$$E_2=E^0 + S\log\gamma + S\log (C+\Delta C) \qquad \textit{Equation 6}$$

The same amount of known concentration of the analyte is added sequentially. The corresponding EMF is represented by Equation 7:

$$E_3=E^0+S\log\gamma+S\log(C+2\Delta C) \quad \text{Equation 7}$$

So, an equation without containing the unknown E^0 and S values can be derived from the equations above [29]:

$$\frac{(E3-E1)}{(E2-E1)}=\frac{log\frac{C+2\Delta C}{C}}{log\frac{C+\Delta C}{C}} \quad \text{Equation 8}$$

From this equation, the concentration (C) is obtained by iteration.

2.5 Ion chromatography

Ion chromatography (IC) was introduced as a new technique for the analysis of inorganic anions and cations by Small in the middle of 1970. IC is based on high performance liquid chromatography (HPLC) which was developed in 1970 with limitation in recognition and monitoring ionic species due to the unsatisfactory detectors. Introduction of conductometric detectors made ionic applications possible. In those the conductivity is the characteristic property of various ionic species in solution and is proportional to the species' concentration [30]. In IC different separation techniques, such as ion-exchange chromatography, ion-exclusion chromatography and ion-interaction chromatography can be used [31]. The separation in ion-exchange chromatography is based on affinity differences between the packed resin materials (in the column) and the ionic species (to be determined). The separation principle of ion-exclusion chromatography is based on Donnan exclusion effect that charged analytes are repelled from the insoluble resin materials in the column containing the similar charge as analytes, thus eluting charged analytes rapidly. The occluded liquid phase in the column retains non-ionic or partially ionized species [31]. In ion-interaction chromatography the problem that ionic solutes (inorganic anions and cations) cannot be or hardly be retained in a stationary phase made of lipophilic materials due to their hydrophilicity when using characteristic reverse-phase eluents, a lipophilic reagent ion with an opposite charge to that of the solute ion is added to the for sequential separation [31].

Development of new detection schemes in IC led to its extended applications and multipurpose use for the analysis of ionic species. By coupling of IC separation system with different detection methods, such as conductivity detection (with/without suppressor), electrochemical detection (amperometry and potentiometry), spectrophotometric detection, or post-column reaction detection, IC analysis can be extended from traditional ionic species to low molecular weight carboxylic acids and low molecular weight organic bases, and also to analyte compounds such as carbohydrates, amino acids, nucleic acids and proteins [31, 32].

More widespread utilization of conductivity detection is assigned to its two advantageous properties. One of them is that all ions possess their unique electrical conductivity, which is determined as a function of concentration. Additionally, simpler construction and operation make conductivity detection superior compared to other detection methods [31]. It was observed that a suppressor column, placed between the ion-exchange analytical column and the detector, could improve the sensitivity of ionic species when the ion detection principle is based on electrical conductance. Detection of target ions can be improved by modifying the liquids (eluent and sample solution) flowing through the suppressor by chemical reduction/ electrochemical suppression of eluent conductivity (background noise) in order to enhance the electrical conductivity of the analyte ions [31].

2.5.1 Instrumentation and principle of ion-exchange chromatography

Ion-exchange chromatography, the ion chromatography technique in which separation is based on the different affinities between the packed resin material in the column and the ionic species to be determined, is suitable for ionic species since only the analyte ions interact with the stationary phase during separation and the stationary phase is tolerant to the sample matrix [33]. Ion-exchange chromatography is a liquid-solid chromatographic method in which the liquid (eluent) flows through the solid stationary phase (cylindrical column) which is packed with uniform small-size ion-exchange particles. In ion-exchange chromatography columns packed with inorganic or organic (polymeric) resin materials (such as polystyrene-divinylbenzene and polymethacrylate) are utilized where charged groups are fixed on their surface [31]. The eluent, easily influenced by ionic strength, pH, temperature, and flow rate, has a significant effect on the retention time of the ions and plays a key role in ionic analysis since the sample is carried by the eluent flowing through the whole chromatographic system

shown in Figure 4. For eluting anions, hydroxide or carbonate based eluents are mostly used [34, 35]. A high-pressure pump is essential to drive the eluent through the column made of stainless steel (generally 5-30 cm long) with controlled flow rate. The sample to be separated and determined is introduced by an autosampler/injector to the flowing eluent stream. To prevent the separation column from contamination and to prolong its lifetime, a guard column packed with the same material as the separation column is installed prior to the separation column. During analysis, different ionic species in the injected sample move at different speed (retention time) through the separation column. This retention is caused by different affinities between the analytes ions and the packed ion-exchange material in the column. Distinct zones assigned to the different affinities are formed and can be detected by the detector. Gaussian peaks are recorded on the chromatogram at different retention times. The efficiency of peak separation is evaluated by the peak shape and symmetry and complete separation between two closely lying peaks. The narrower and the more symmetric the peaks are, the better is the separation efficiency [30].

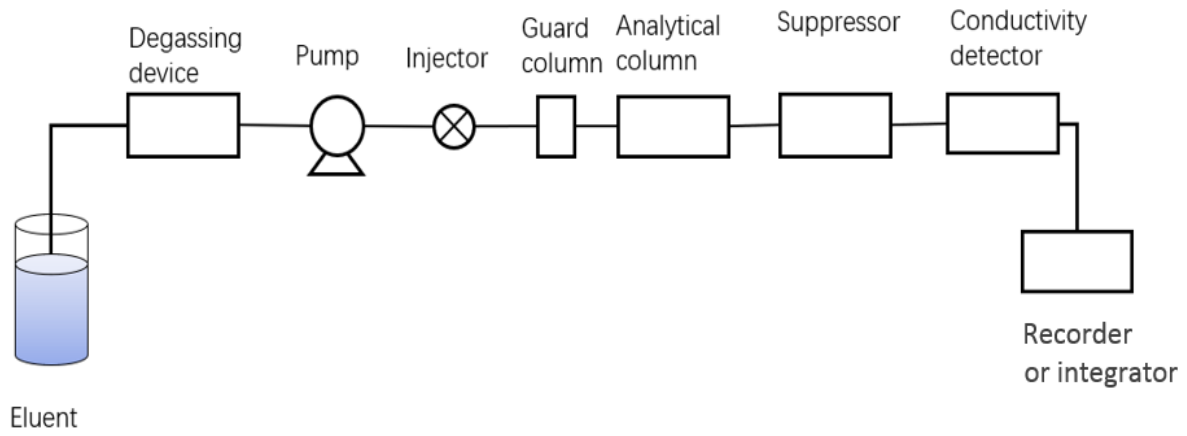

Figure 4. Diagram of instrumental components used for suppressed ion chromatography with a conductivity detector.

2.5.2 Sample preparation procedure

There are some predefined sample preparation methods which help to minimize the influence of the coexisting sample matrix and to prevent blocking or degeneration of the column and to provide a more reliable and sensitive analysis. Dilution of sample is desirable when high concentrations of sample ions are determined because otherwise the column would be

overloaded. Filtration of the sample (sufficient for most applications) is performed to remove particles and microorganisms in samples to prevent blocking and contamination of columns and flow lines [4].

2.5.3 Standard addition method suitable for use in IC applications

A standard addition method is used when the unknown sample is measured in presence of a complicated matrix with high-potential interference on the analytical signals. Deduction from the increased signal, after the addition of a fixed amount of a known concentration of analyte to the sample, the initial concentration in the unknown sample can be calculated according to the response analysis between the concentration of diluted standard and its corresponding signal. As the unknown sample is measured with all diluted standards, the linear response is extrapolated towards zero and the intercept on x-axis gives the initial concentration of the sample [36]. It is advantageous to use this method for correction of the matrix effect since sample matrix influences both the calibration standards and the sample similarly, especially for biological samples. However, the sample consumption is higher in standard addition method than when the calibration is made by separate standard solutions and it is a labor-intensive process as more efforts is required to prepare the calibration plot for each sample separately [37].

3. Experimental section

3.1 Chemicals

Synthesis of the acetate-selective ionophore used in this study was made as described in a previous study [38] made at Tartu University. The monomer 3,4-ethylenedioxythiophene (EDOT>97%), 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES), potassium chloride (KCl, ≥99%), ethanol, D-(-)-tartaric acid (99%), L(+)-lactic acid lithium salt and sodium carbonate (Na_2CO_3) were obtained from Sigma-Aldrich. Selectophore grade polyvinylchloride (PVC) of high molecular weight, tridodecylmethylammonium chloride (TDMACl), 2-nitrophenyl octyl ether(o-NPOE), succinic acid, citric acid anhydrous, sodium

formate and tetrahydrofuran (THF, >99.5%) were purchased from Fluka. Sodium acetate (CH_3COONa) was purchased from Merck. Sodium bicarbonate ($NaHCO_3$), nitric acid (HNO_3) and sulfuric acid (H_2SO_4) were obtained from J.T.Baker. L(-)-malic acid was obtained from Acros Organics. The wine (Nederburg, South Africa) was purchased from an alcohol shop (Alko) in Turku, Finland. All chemicals used were of analytical-reagent grade. Milli-Q water (resistivity 18.2 MΩcm) was used to prepare all solutions. The calibration standard solutions and spiking solutions were prepared by dissolving sodium acetate in Milli-Q water.

3.2 Electrochemical synthesis of PEDOT(Cl) as solid contact

Prior to electropolymerization, five glassy carbon (GC) electrodes (further referred to as E1, E2, E3, E4 and E5 in graphs and tables) were well polished using Al_2O_3 (0.3 μm). Chemical cleaning was carried out in 1 M HNO_3 for 1 min and ultrasonic cleaning was done both in ethanol and water for 30 min. PEDOT(Cl) was electrochemically deposited on the surface of the polished glassy carbon electrodes by galvanostatic electropolymerization in the one-compartment three-electrode electrochemical cell.

PEDOT was synthesized in a solution containing 0.01 M EDOT monomer and 0.1 M KCl as supporting electrolyte. The EDOT-KCl solution was purged with nitrogen for 20 min prior to electropolymerization. A GC disk electrode (area = 0.07 cm^2) was used as working electrode, a GC rod electrode as counter electrode, and a commercial single-junction Ag/AgCl (3 M KCl) electrode as reference electrode. A constant current of 0.014 mA (0.2 mA/cm^2) was applied to the electrochemical cell for 714 s resulting in *ca.*10 mC charge consumed during polymerization of EDOT on the surface of the working electrode.

3.3 Cyclic voltammetric characterization of the PEDOT film

Cyclic voltammetric characterization of the GC electrodes was carried out in the one-compartment three-electrode electrochemical cell using an Autolab instrument (AUTO30.FRA2-Autolab Eco Chemie, B.V., the Netherlands) and Nova 2.1 software. The arrangement of electrodes was similar to the electropolymerization process (explained above).

Each GC electrode was characterized in deaerated 0.1 M KCl solution within the potential range from -0.5 V to 0.5 V and three scans (scan rate 100 mV/s, step potential 0.00244 V). The same procedure was applied to all five electrodes before and after the galvanostatic polymerization of PEDOT on the electrode surface to study the PEDOT(Cl) film.

3.4 Fabrication of solid-contact acetate-selective ISEs

The fabrication procedure of solid-contact acetate-selective ISEs was similar to the studies of Mousavi et al. [6] while with a different recipe. The acetate-selective membrane cocktail was prepared by dissolving the acetate-selective ionophore (2 wt %) with o-NPOE (65 wt %), PVC (33 wt %), and TDMACI (50 mole % relative to the ionophore) in THF (dry content *ca*. 17 %). Drop casting of 100 µl (2 x 50 µl) membrane cocktail was performed on the surface of dried GC/PEDOT(Cl) electrodes. After two-day evaporation of the solvent (THF) from the ISM, all GC/PEDOT (Cl)/acetate-ISM electrodes were conditioned in 0.1 M CH_3COONa solution for three days before further use.

3.5 Electrochemical impedance spectroscopy characterization of acetate-selective ISEs

The impedance of acetate-ISEs was investigated using an Autolab General Purpose Electrochemical System equipped with Frequency Response Analyzer System (AUTO30.FRA2-Autolab Eco Chemie, B.V., the Netherlands) and FRA software. The EIS measurements were performed in the same three-electrode electrochemical cell mentioned above. EIS characterization was done before and after drop-casting of the acetate-selective ionophore membrane in 0.1 M sodium acetate solution. The coated membrane was characterized by engaging EIS at open circuit potential within the frequency range between 10 mHz and 100 kHz at the excitation potential of 10 mV amplitude.

3.6 Potentiometric measurements

A 16-channel potentiometer (Lawson Labs, Inc) was used for potentiometric measurements connected to a desktop computer for data acquisition. The potentials of five GC/PEDOT(Cl)/acetate-ISM electrodes were measured simultaneously against the single junction Ag/AgCl (3 M KCl) reference electrode in standard sodium acetate solutions within the concentration range of 10^{-1} – 10^{-8} M. An automatic dilution system was utilized for serial tenfold dilution of the 0.1 M standard acetate solution. The pH of 20 ml wine sample (initial pH = 3.56) was adjusted to 6 by 0.1 M HEPES buffer solution (pH = 7) and diluted to 100 ml using Milli-Q water. The quantification of acetate in the wine sample was done by following both the standard single-addition method (one addition of 1 ml of 1 M CH_3COONa to 50 ml pretreated wine sample) and the standard double-addition method (serial additions of 1 ml of 1 M CH_3COONa to 50 ml pretreated wine sample) to get rid of the matrix effect resulting from the complicated wine matrix. The potential was measured before and after each standard addition for 5 min. All experiments were performed at room temperature.

3.6.1 Selectivity coefficient measurement

By the means of the separate solution method (SSM), the selectivity coefficients ($\log K_{acetate}^{pot}$) of for acetate and the interfering ions were determined in 10^{-1} M their salt solutions potentiometrically. 50 ml of 10^{-1} M succinate, tartrate, malate, citrate, formate, acetate and lactate solutions were prepared. The reaction cell included five acetate-selective electrodes and a reference electrode. The EMF was recorded after a five-minute stabilizing time in 10^{-1} M salt solution and a tenfold dilution was carried out by an automatic dilution system after that. Then the EMF was recorded again in 10^{-2} M solution of the same salt. The same procedure was repeated for all the salts mentioned above.

3.7 Ion chromatographic instrumentation

The ion chromatographic system from Metrohm consists of an 818 IC Pump, an 820 IC Separation Centre, an 833 IC liquid handling Unit, an 830 IC Interface, and a 732 IC conductivity detector. The anion separation was performed in suppressor mode (with chemical suppression) using a Metrosep Anion Dual 2 analytical column (technical information shown in Table 1) connected with a Metrosep RP 2 guard/3.5 column and a 750 autosampler. Eluent containing the mixture of 0.6 mM Na_2CO_3 and 2 mM $NaHCO_3$ with 0.1 ml/min flow rate was used for 60 min recording time [39]. 10 mM H_2SO_4 was used as the regenerant of the suppressor. 20-µl injection volume was used for analysis.

Table 1. Technical information of Metrosep Anion Dual 2 column in use.

Substrate	Polymethacrylate with quaternary ammonium groups
Column dimensions	75 x 4.6 mm
Column body	Stainless steel
Standard flow	0.8 mL/min
Maximum pressure	7 MPa
Particle size	6 µm
pH range	1 - 12
Organic modifier	0 - 20%
Capacity	17 µmol (Cl^-)

Standard stock solution of sodium acetate was prepared by weighing an adequate mass of sodium acetate and dissolving it in Milli-Q water in a volumetric flask and filling to the mark to obtain the concentration of 10^{-1} M. Standard solutions containing different acetate concentrations (0.01, 0.05, 0.1, 0.5 mM) were prepared by diluting the stock solution for the calibration. All prepared solutions were stored in plastic bottles for further use. Filtration of the samples was performed before injection with a 0.45-µm pore size polydisc inline-filter (Whatman™, UK).

Additionally, 10^{-3} M succinate, malate, citrate, tartrate, lactate, and formate solutions were prepared and measured individually with IC using the optimized parameters and the eluent mentioned above. This was done in order to determine the retention times of possible interfering ions in wine.

3.8 Standard addition for the determination of acetate by IC

A standard addition method was used to determine the unknown concentration of acetate to reduce the effect of the wine matrix on the measurement results. 100 µl of the filtered sample was diluted 500 times with Milli-Q water. An equal amount (5 ml) of the diluted wine sample was put into five different volumetric flasks of 10 ml each. To each flask, 0 mM, 0.05 mM, 0.1 mM, 0.15 mM, 0.2 mM sodium acetate standard solution was added and filled to the mark with Milli-Q water. In this way, each flask contained the same concentration of the wine sample (diluted 1000 times from original wine) and different concentrations of the standard (0 – 0.2 mM). For each flask, a measurement of the analytical signal was then recorded. The analytical signal versus the concentrations of diluted standard was plotted. From the plot, the x-intercept gives the concentration of analyzed sample and by correcting the value with the dilution factor (1000), the acetate concentration in wine was determined [36].

4. Results and discussion

4.1 Galvanostatic electropolymerization

Galvanostatic electropolymerization of PEDOT was studied by chronopotentiometric curves (Figure 5) in a monomer solution containing 0.01 M EDOT and 0.1 M KCl as supporting electrolyte with 0.2 mA/cm^2 current density as studied by Bobacka et al. [19]. The potential initially rose to over 1.0 V, then decreased and gradually stabilized at *ca.* 0.9 V. High potential in the beginning can be explained by a higher oxidation potential of the monomer needed to produce radical cations compared to the following oxidation of the dimers which need a lower potential and therefore the potential decreases [25].

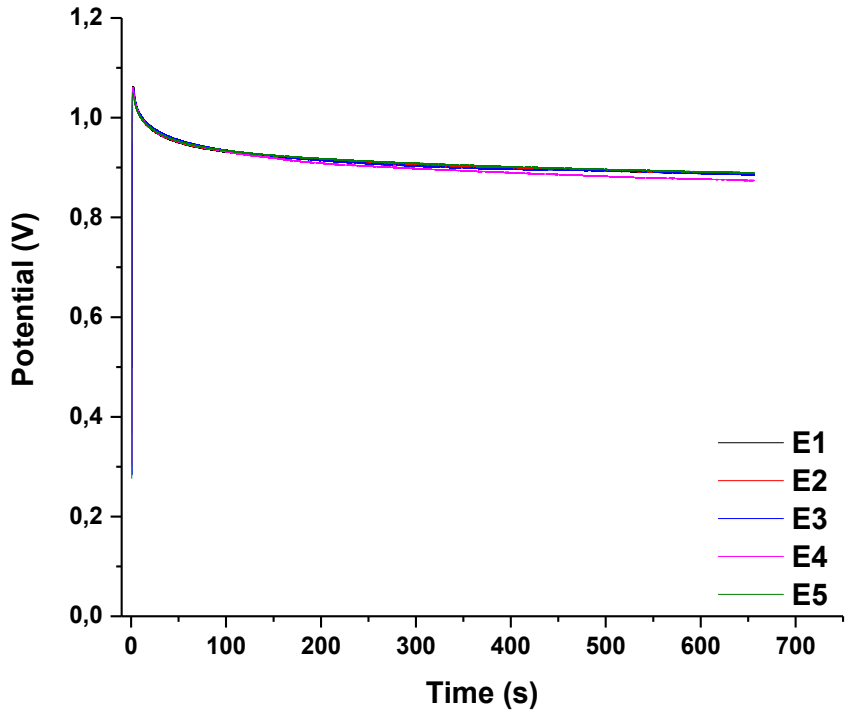

Figure 5. Chronopotentiometric curves of five GC electrodes recorded during galvanostatic electropolymerization in the solution containing 0.01 M EDOT and 0.1 M KCl as supporting electrolyte. (Current density = 0.2 mA/cm^2)

4.2 Cyclic voltammetry

Cyclic voltammograms, used to characterize the deposited PEDOT(Cl) film and to study the electrochemical properties of the GC/PEDOT(Cl) electrode before and after the galvanostatic electropolymerization, were recorded and one of them is shown in Figure 6 as an example. The same procedure and parameters as used in CV for characterization were applied to all GC electrodes and GC/PEDOT(Cl) electrodes. An insignificant current (*ca.* 0.4 μA) before the galvanostatic electropolymerization process was observed within the working potential range (-0.5 V – 0.5 V), while an obvious capacitive-like current was observed after PEDOT(Cl) polymerization process at the GC/PEDOT(Cl) electrode; similar to the observation by Bobacka et al. [19]. The noticeable current recorded indicates redox capacitance resulting from the synthesized PEDOT(Cl) film during galvanostatic electropolymerization [6].

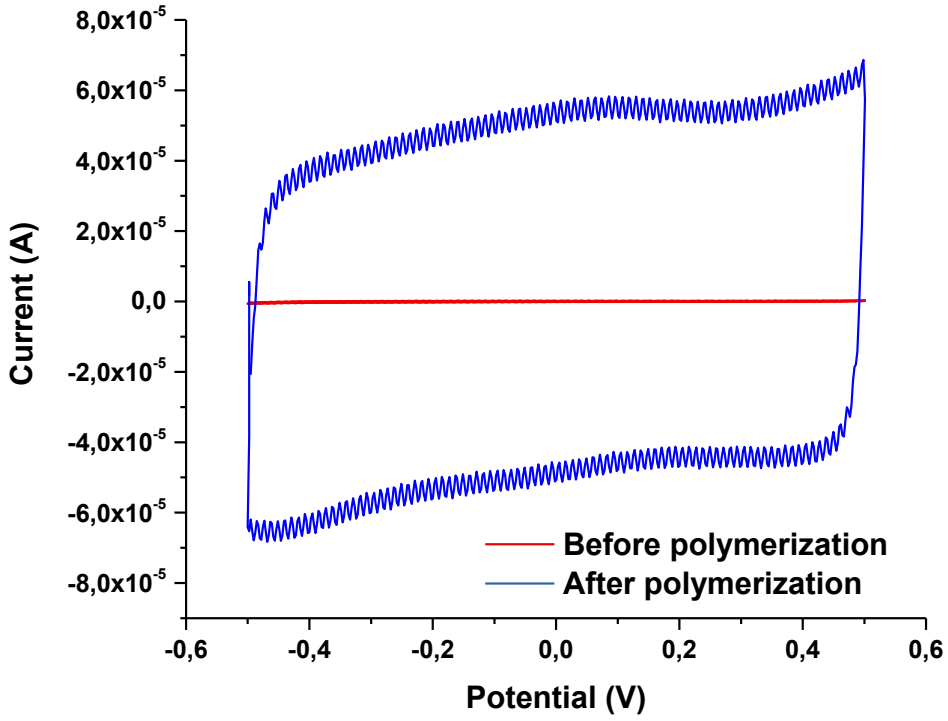

Figure 6. Cyclic voltammograms (the 3rd scan) of bare GC 1 (*red line*), GC/PEDOT(Cl) (*blue line*) recorded in 0.1 M KCl solution at a potential scan rate of 0.1 V/s.

4.3 EIS measurements

The impedance spectra of five GC/PEDOT(Cl) electrodes (without ISM) recorded in 0.1 M CH_3COONa solution are shown in Figure 7. The shape of the impedance spectra is similar to the PEDOT layer in KCl electrolyte studied by Bobacka et al. [19]. Vertical capacitive lines of 90° angle were found and were consistent with Bobacka's study. Capacitive lines are extended from high frequency (100 kHz) to extremely low frequency (10 mHz) with capacitor-like performance which indicates that the redox process occurs throughout the entire PEDOT(Cl) film. The absence of a semicircle in the high frequency range in Figure 7 can be explained by the fast charge-transfer kinetics [40]. The low-frequency capacitance (C_L) of GC/PEDOT(Cl) can be estimated by using the following equation $C_L = \frac{1}{2\pi f(-Z'')}$, where f is the lowest frequency used to record the spectra (10 mHz), and -Z" is the imaginary part of the impedance at this frequency. The calculated redox capacitance C_L of GC/PEDOT(Cl)

electrodes was ca. 292 µF, 357 µF, 376 µF, 372 µF and 353 µF, respectively. The corresponding solution resistance (R_S) was *ca.* 250.16 Ω, 230.65 Ω, 218.82 Ω, 224.73 Ω and 233.33 Ω, respectively.

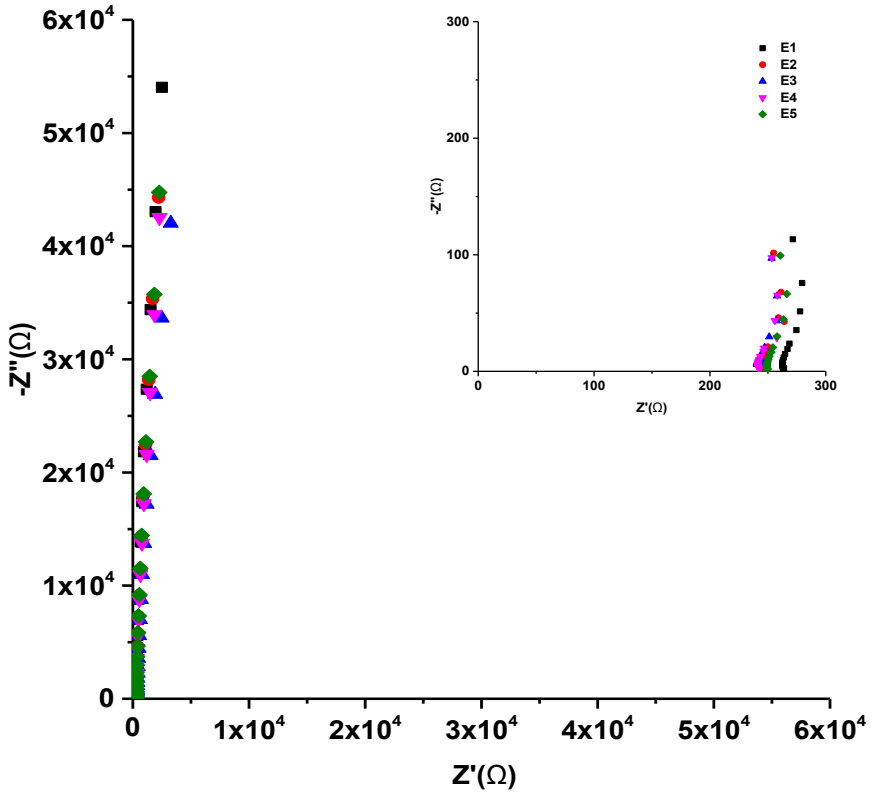

Figure 7. Impedance plots of five GC/PEDOT(Cl) electrodes in a deaerated solution containing 0.1 M CH_3COONa as supporting electrolyte and magnification of the high frequency region (from 100 kHz to 1259 Hz).

The impedance spectra of five GC/PEDOT(Cl)/acetate-selective electrodes were recorded in 0.1 M CH_3COONa solution as shown in Figure 8. Accompanying by diffusion lines at low frequency, semicircles were observed at high frequency. The formation of semicircles is attributed to bulk resistance and capacitance of ISM arising from the geometry. The observed diffusion lines can be assigned to ion diffusion throughout the PEDOT and ISM layers [40].

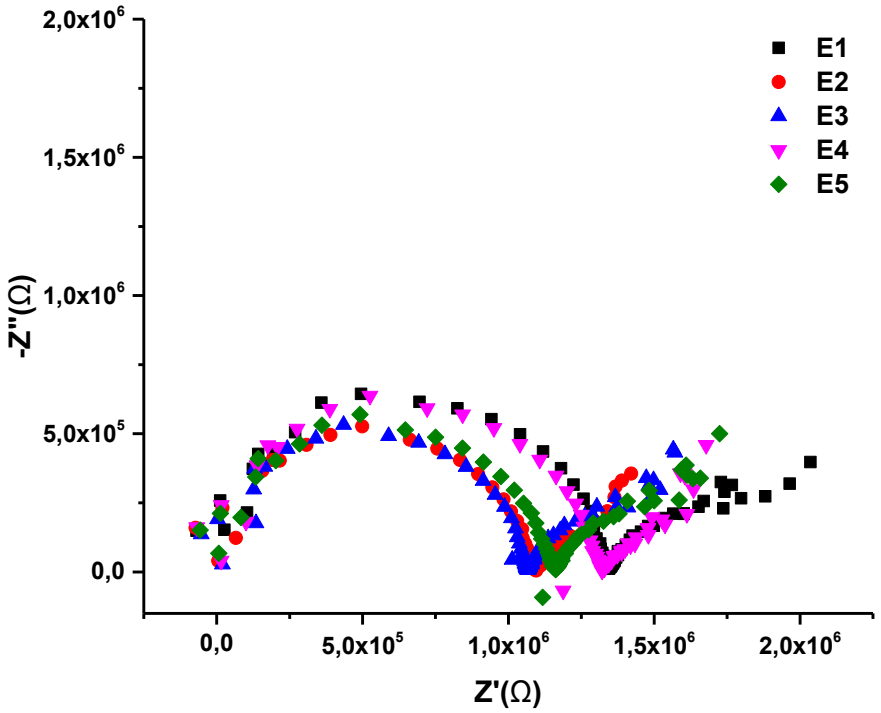

Figure 8. Impedance plots of five GC/PEDOT(Cl)/ISM electrodes in a deaerated solution containing 0.1 M CH_3COONa as supporting electrolyte.

The bulk resistance values (R_b) vary between 1.0 and 1.3 MΩ as shown in Table 2. Differences in the bulk resistance can be due to slightly different thickness of the ISM layers in five electrodes. For example, GC/PEDOT(Cl)/acetate-selective ISE 1 has the thickest film while GC/PEDOT(Cl)/acetate-selective ISE 3 has the thinnest film among the five replicates. In parallel, geometric capacitance values (C_g) of ISMs can be deduced from the frequency value (f_{max}) at the top of the semicircle. Capacitance values (C_g) can be calculated by using equation $C_g = \frac{1}{2\pi f_{max} R_b}$ and numerical values are shown in Table 2.

Table 2. Numerical values of bulk resistance (R_b) and geometric capacitance (C_g) from obtained impedance data.

No. of acetate ISEs	Bulk resistance R_b (MΩ)	Geometric capacitance C_g (pF)
E1	1.328	9.516
E2	1.076	11.745
E3	1.046	12.083
E4	1.308	9.669
E5	1.139	11.102

4.4 Potentiometric response of the solid-contact acetate-selective ISEs

Calibration curves for the acetate-selective electrodes were recorded at two different times. Potentiometric responses of five freshly-made GC/PEDOT(Cl)/acetate-ISM electrodes were investigated in standard sodium acetate solutions and the EMF values versus logarithm values of the acetate activity in the wine sample were plotted and as shown in Figure 9.

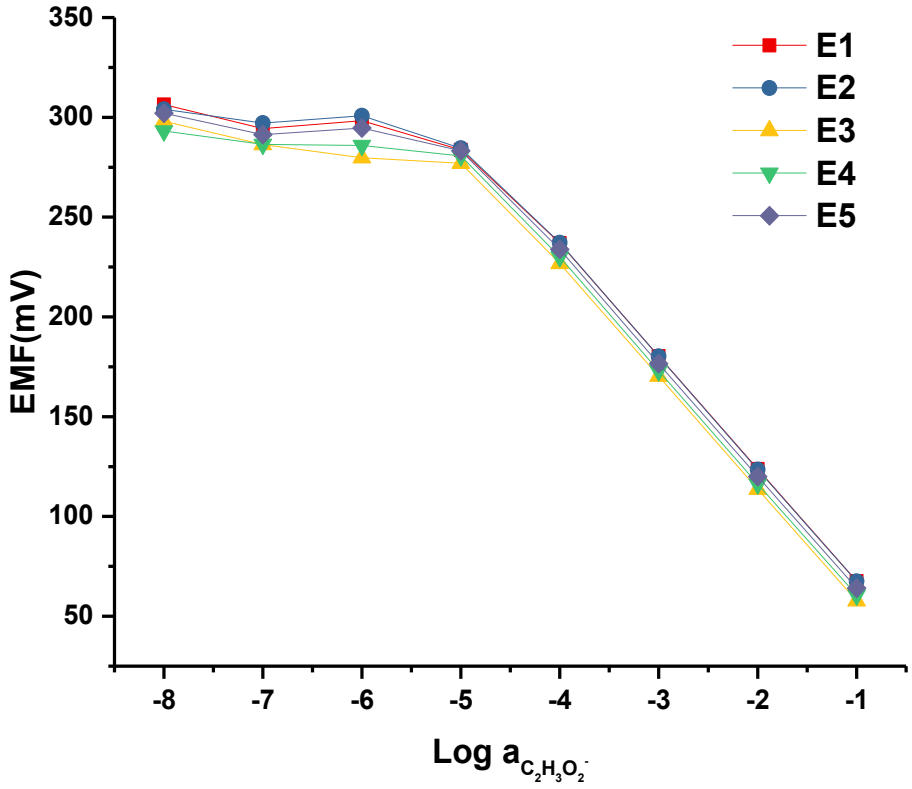

Figure 9. Calibration curves of five freshly fabricated GC/PEDOT(Cl)/acetate-ISM electrodes in standard acetate solutions.

The average slope of the calibration curves in the linear working range (10^{-1} M – 10^{-5} M) with the five acetate-selective electrodes (E1 – E5) was -55.01 ± 0.34 mV/decade (average value ± standard deviation (SD)) which is close to Nernstian response of ISEs with the detection limit of 10^{-5} M as shown in Table 3.

Table 3. Potentiometric calibration data of five GC/PEDOT(Cl)/acetate-ISM electrodes. (SD: standard deviation)

No. of acetate ISEs	E1	E2	E3	E4	E5	Average ± SD
Standard potential E^0 (mV)	67.21	67.23	57.07	62.11	63.95	63.52 ± 4.22
Slope (mV/decade)	-54.56	-54.77	-55.14	-55.38	-55.21	-55.01 ± 0.34

Calibrations of the same five acetate-selective electrodes (E1 – E5) were investigated after three weeks of conditioning in 0.1 M standard sodium acetate solution. The EMF values versus logarithm values of the acetate activity in the wine sample were plotted and as shown in Figure 10.

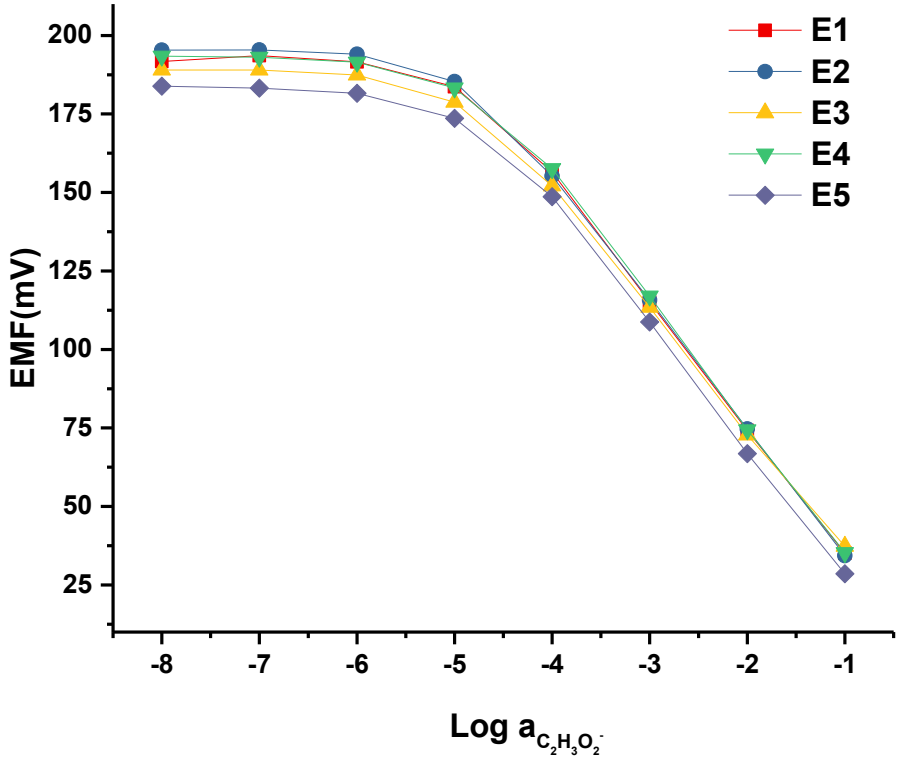

Figure 10. Calibration curves of five GC/PEDOT(Cl)/acetate-ISM electrodes in standard acetate solutions after conditioning in 0.1 M CH_3COONa for three weeks.

The mean slope of calibration curves in the linear range (10^{-1} M – 10^{-4} M) was found to be -42.99 ± 0.84 mV per decade (average value ± standard deviation (SD)) and as shown in Table 4. The detection limit for the fabricated acetate-selective ISEs was 10^{-5} M. The decrease in slope may be due to leakage of the ionophore from membrane during storage of the electrodes. Therefore, the acetate measurements conducted on the wine sample were carried out using these electrodes.

Table 4. Potentiometric calibration data of five freshly fabricated GC/PEDOT(Cl)/acetate-ISM electrodes.

No. of acetate ISEs	E1	E2	E3	E4	E5	Average ± SD
Standard potential E^0(mV)	-4.87	-4.40	-2.33	-0.86	-5.26	-3.54 ± 1.878
Slope (mV/decade)	-43.17	-44.07	-43.41	-42.33	-41.99	-42.99 ± 0.836

4.4.1 Standard addition methods for ISEs

The acetate concentration in the wine sample was measured and calculated by standard single-addition method (method 1) and standard double-addition method (method 2) with five acetate-selective electrodes as shown in Table 5. The concentration of acetate in the wine sample was 0.0572 M using method 1 and 0.0947 M using method 2. The reproducibility of both calibration methods was good with standard deviations of 0.0059 M for method 1 and 0.0003 M for method 2 using five acetate-selective electrodes. The concentration difference from the two methods can be linked to their different assumptions. In method 1, the slope of the calibration curve using the standard solutions is taken into consideration when calculating the concentration of unknown sample. However, method 2 does not consider the slopes, since the slopes might be different in the standard solutions and in the unknown sample.

Table 5. Sample concentration measured by five acetate-selective electrodes using single-addition standard method and double-addition standard method and corresponding mean sample concentration values and standard deviations (SD).

No. of acetate ISEs	E1	E2	E3	E4	E5	Average ± SD
original *C* (M) (method 1)	0.0614	0.0605	0.0495	0.0522	0.0623	0.0572 ± 0.0059
original *C* (M) (method 2)	0.095	0.0947	0.0943	0.0945	0.0951	0.0947 ± 0.0003

4.4.2 Measurements of selectivity coefficients

The selectivity coefficient ($\log K_{acetate}^{pot}$) for each interfering ion *j* existing in wine was determined by the separate solution method (SSM) [41]. The activity coefficients of the primary ion *i* (acetate) and the interfering ion *j* were calculated by the Debye Hückel equation and the slope (S_{exp}) was calculated from the experimental data. The selectivity coefficients were calculated using the extended Nikolskii equation (Equation 9):

$$logK_{acetate}^{pot} = \frac{E_j - E_i}{S_{exp}} + (1 - \frac{z_i}{z_j})\, loga_i \qquad \textit{Equation 9}$$

where E_i and E_j are the measured EMF potentials for a solution containing only the salt of the acetate ion *i* with charge z_i = -1 or the interfering ion *j* with charge z_j, respectively. a_i is the activity of the acetate ions in the sample.

The logarithms of the selectivity coefficients for the acetate-selective electrodes were calculated and are shown in Table 6. Formate and lactate have the highest selectivity

coefficients, which means that they are the main interfering anions to the acetate determination in wine.

Table 6. The selectivity coefficients of various interfering anions in wine for acetate-ISEs. (Average values ± standard deviation values for five acetate-ISEs)

Ion j	Succinate	Tartrate	Malate	Citrate	Formate	Lactate
$\log K^{pot}_{acetate,j}$	-2.24 ± 0.09	-3.84 ± 0.14	-3.32 ± 0.12	-3.08 ± 0.11	-0.80 ± 0.03	-0.84 ± 0.05

4.5 IC measurements

4.5.1 IC method development

Chemically suppressed ion-exchange chromatography coupled to a conductivity detector was used for determination of the acetate content in wine. After choosing the separation column and the detector, an initial eluent composition of 2.0 mM $NaHCO_3$ and 1.3 mM Na_2CO_3 and flow rate of 0.7 ml/min recommended by Metrohm were used. Using the recommended operating conditions, the peak separation was not ideal as shown in Figure 11.

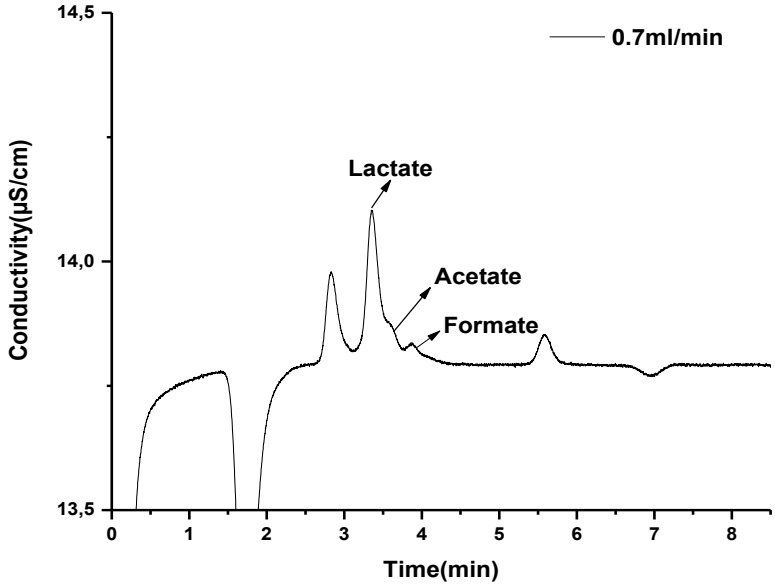

Figure 11. Ion chromatogram of 1000 diluted wine sample using recommended operational conditions (Flow rate: 0.7 ml/min, eluent: 2 mM $NaHCO_3$ and 1.3 mM Na_2CO_3, run time: 40 min.).

Slowing down the flow rate is one solution to improve the peak separation. Flow rates of 0.5 ml/min and 0.3 ml/min were applied to diluted wine sample (dilution factor 1000). However, at these flow rates, there was still peak overlap of acetate and lactate, as shown in Figure 12.

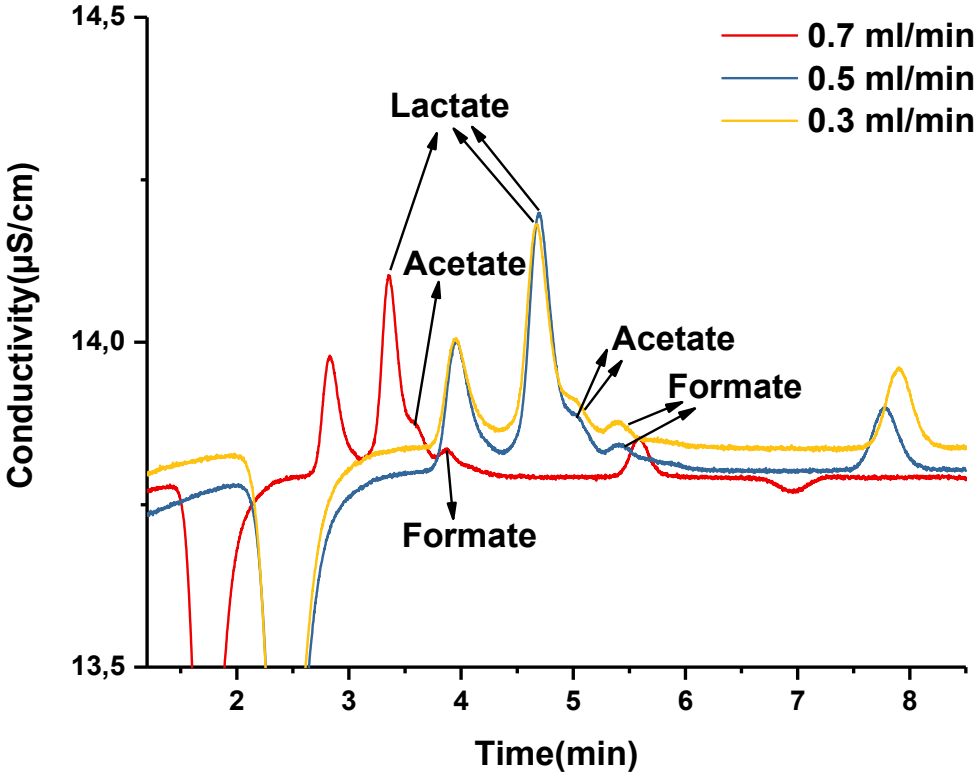

Figure 12. Comparison of chromatograms with different flow rates (0.3, 0.5 and 0.7 ml/min) using the eluent mixture of 2.0 mM $NaHCO_3$ and 1.3 mM Na_2CO_3.

Next, eluent composition was altered in order to obtain a longer retention time for acetate by decreasing the concentration of Na_2CO_3 to 0.6 mM from 1.3 mM as shown in Figure 13.

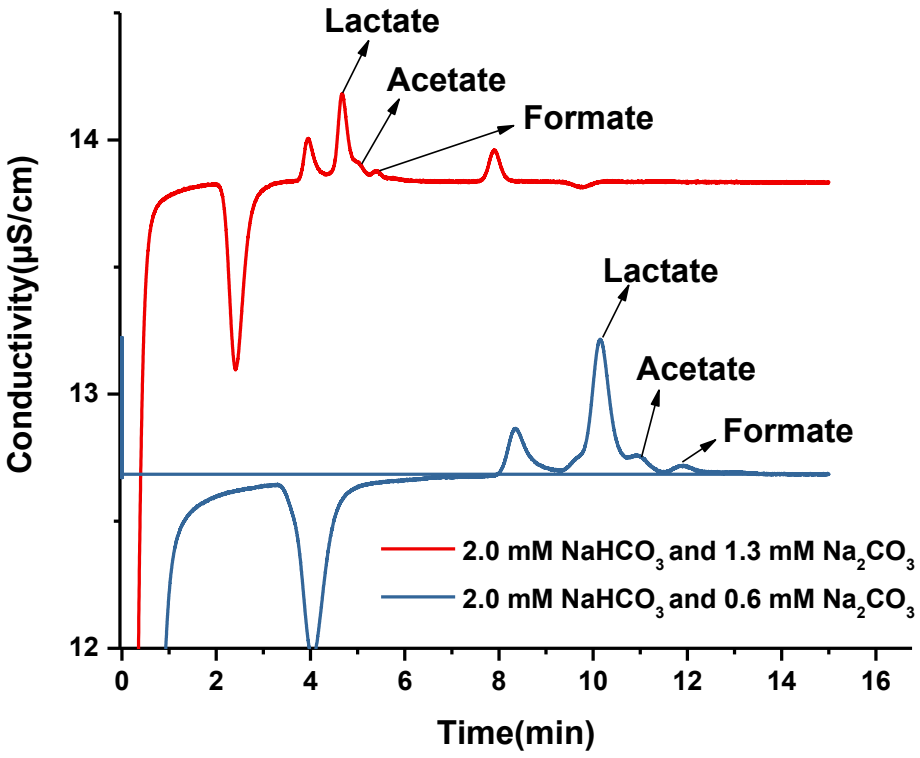

Figure 13. Comparison of chromatograms with the same flow rate (0.3 ml/min) using eluent mixture of 2.0 mM $NaHCO_3$ and 1.3 mM Na_2CO_3 (red line) and 2.0 mM $NaHCO_3$ and 0.6 mM Na_2CO_3 (blue line).

A further trial with the same eluent mixture of 2.0 mM $NaHCO_3$ and 0.6 mM Na_2CO_3 varying the flow rate from 0.3 ml/min to 0.1 ml/min was carried out and the result is shown in Figure 14. As can be seen in Figure 14, the separation of the acetate peak from the lactate and formate peaks was still improved by using the 0.1 ml/min flow rate. Although temperature has effect on the retention time, there is no possibility to change the temperature in the current IC system. Therefore, the operational parameters used in this work were 0.1 ml/min flow rate and eluent composition of 2.0 mM $NaHCO_3$ and 0.6 mM Na_2CO_3 at room temperature.

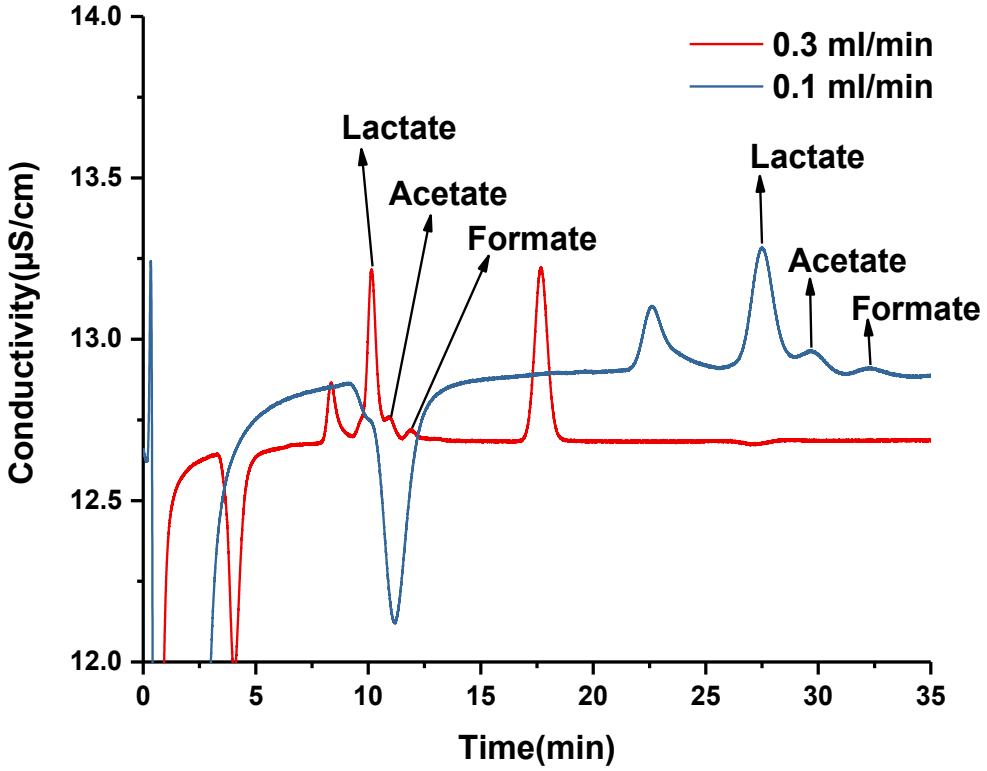

Figure 14. Comparison of chromatograms using eluent mixture of 2.0 mM $NaHCO_3$ and 0.6 mM Na_2CO_3 with different flow rates of 0.3 ml/min (*red line*) and 0.1 ml/min (*blue line*).

In addition to lactate and formate, the retention times of succinate, malate, citrate, and tartrate were measured in their standard solutions at concentration of 10^{-3} M to confirm no overlapping between their peaks and the peak of acetate, which is of interest. The retention times of all interfering ions and the main ion (acetate) is shown in Table 7.

Table 7. Retention times of the main ion (acetate) and the interfering ions as measured by IC in their standard solutions at concentration of 10^{-3} M.

Ions	Succinate	Malate	Citrate	Tartrate	Lactate	Acetate	Formate
Retention time(min)	39.09	41.02	41.72	42.12	45.00	47.90	53.64

The retention times of lactate and formate are closest to that of acetate, which may easy contribute to the overlap with the peak of acetate, resulting in a bigger area of the peak of

acetate. Thus, lactate and formate have high potential to interfere in acetate determination while analyzing wine with IC.

4.5.2 Calibration of IC using standard sodium acetate solutions

The peak area of the acetate signal (µS/cm) versus concentration of sodium acetate standards (mM) was plotted and a linear relation within the concentration range of 0.01 mM – 0.5 mM was obtained (Figure 15). The average slope of calibration curves using the standard acetate solutions in IC was 625.02 ± 6.049. In addition, the average intercept value obtained was -1.09 ± 1.550. The regression coefficient (r^2) value, representing the linearity of response, of this calibration curve was 0.9998, which indicates an excellent suitability of the IC system.

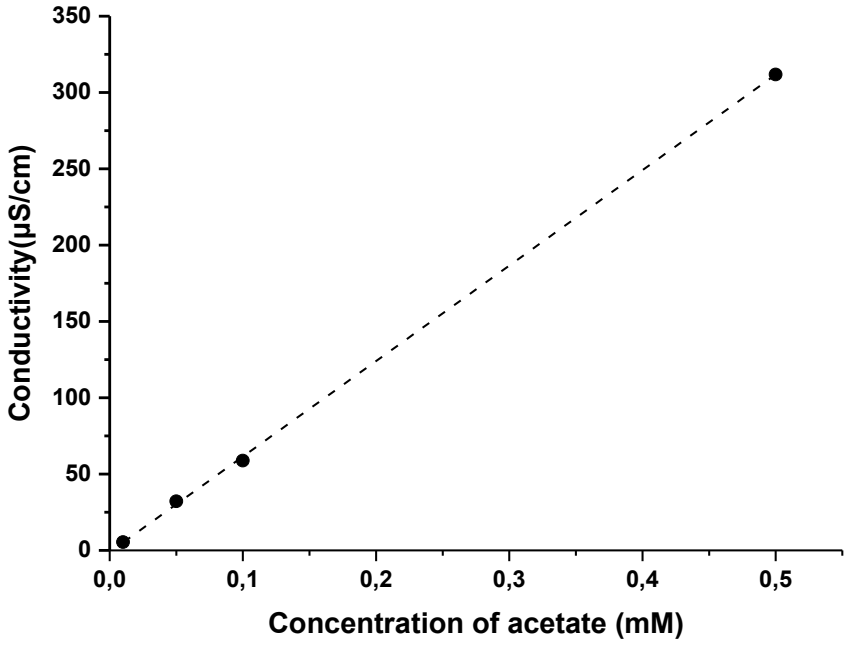

Figure 15. Calibration curve of sodium acetate with concentrations of 0.01, 0.05, 0.1 and 0.5 mM using eluent containing 2 mM $NaHCO_3$ and 0.6 mM Na_2CO_3 with 0.1 ml/min flow rate.

The repeatability of retention time and the analytical signal using the standard acetate solutions (6 injections for each concentration), representing the precision of the method, was studied and the mean values of the retention times and the analytical signals as well as their RSD values are shown in Table 8. The obtained RSD values were below 5 %, which signifies

a good repeatability. The concentration of acetate in the wine sample with a combined uncertainty was 0.0085 ± 0.0013 M obtained when this calibration method was used and after correcting the value with the dilution factor.

Table 8. Repeatability of retention times and the analytical signals observed for the standard acetate solutions (6 injections for each concentration). (RSD: relative standard deviation)

Concentration of acetate (mM)	Mean retention time (min)	RSD (%)	Mean area of the signal (μS/cm)	RSD (%)
0.01	29.38	0.36	5.45	4.79
0.05	29.38	0.13	32.19	0.71
0.1	29.45	0.08	58.77	0.41
0.5	29.66	0.13	311.74	0.22

4.5.3 IC-standard addition method

The chromatogram of conductivity responses against retention times of different concentrations of the diluted standard acetate (in wine sample) is shown in Figure 16.

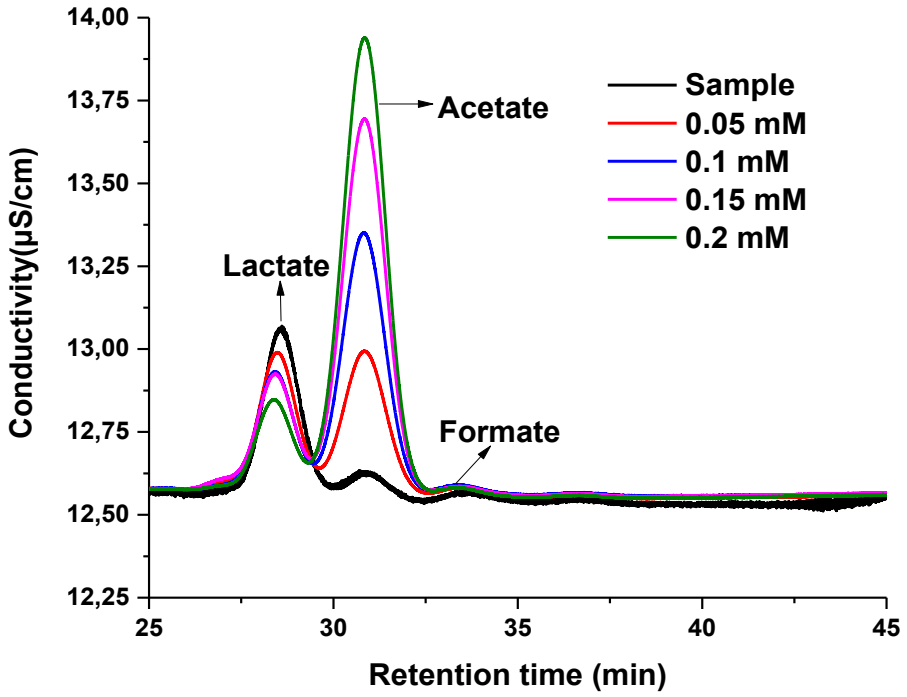

Figure 16. Conductivity response after the standard addition method using 0, 0.05, 0.1, 0.15 and 0.2 mM standard sodium acetate added to diluted wine sample (dilution factor:1000). (Eluent: 2 mM $NaHCO_3$ and 0.6 mM Na_2CO_3, flow rate: 0.1 ml/min)

From the chromatogram, the peak area of the acetate signal (µS/cm) versus concentration of sodium acetate standards (mM) added to the wine sample was plotted and a linear relation between conductivity response and the added standard sodium acetate concentrations of 0 mM, 0.05 mM, 0.1 mM, 0.15 mM and 0.2 mM was obtained as shown in Figure 17.

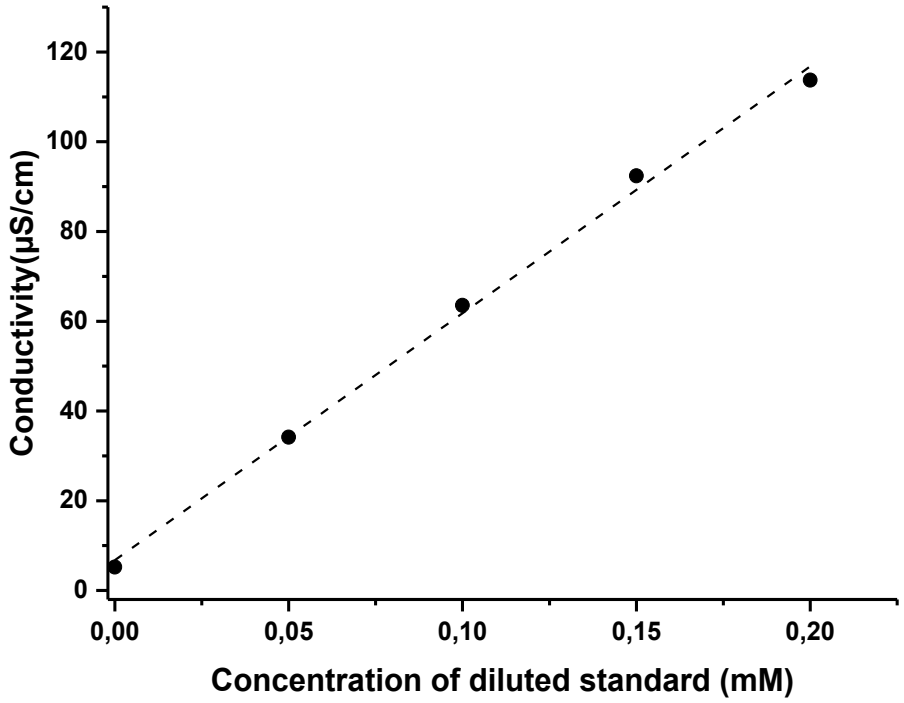

Figure 17. Calibration curve of standard addition method using 0.01, 0.05, 0.1 and 0.5 mM standard sodium acetate added to diluted wine sample (dilution factor:1000). (Eluent: 2 mM $NaHCO_3$ and 0.6 mM Na_2CO_3, flow rate: 0.1 ml/min)

The average slope of calibration curves using standard addition method in IC was 550.51 with a standard deviation value of 18.14. In addition, the average intercept value obtained was 6.7558 with a standard deviation value of 2.222. The regression coefficient (r^2) value of this calibration curve was 0.9968, which indicates an excellent suitability of the IC system. The concentration of acetate in the same wine sample with a combined uncertainty was 0.0123 ± 0.0041 M obtained with this calibration curve using the standard addition method for correcting the matrix effect in the wine sample and after correcting the value with the dilution factor.

4.6 Comparison of the results by using both techniques

The concentration of acetate in the same wine sample was measured by both techniques, i.e. acetate-selective electrodes and IC. The acetate content was also investigated for the legal limit (i.e. 1.4 g/L or *ca.* 0.0233 M) of acetic acid content in red wine.

With IC, the concentration of acetate in the wine sample with a combined uncertainty value was found to be 0.0123 ± 0.0041 M by using the standard addition method for correction of the matrix effect and 0.0085 ± 0.0013 M when the conventional calibration curve without correction of the matrix effect was used. Since the acetate concentrations in the wine sample obtained by IC using both calibration methods were below the legal limit of 0.0233 M, no spoilage of the wine used in the study could be signified.

The acetate concentration of the wine sample, using the single-addition method, was measured to be 0.0572 M with a standard deviation value of 0.0059 M when five acetate-selective electrodes were used. When the double-addition method was used the acetate concentration in the wine sample was found to be 0.0947 M with a standard deviation value of 0.0003 M. A comparison of these values with the upper legal limit of 0.0233 M shows spoilage of the wine sample since the acetate concentrations obtained using both standard addition methods with the potentiometric acetate-selective electrodes exceed the upper limit.

The concentration difference between the two techniques is significant. One reason for the difference might be leaching of the lipophilic ionophore from the acetate-selective membrane into the aqueous solution. It was observed that the conditioning solution where the ISEs were kept got unclear with time and the acetate measurements in the wine sample were made after three weeks of preparation and conditioning of the ISEs. Leakage from PVC-based ISMs (PVC as membrane polymer matrix) has been observed for plasticizer [42], ionophore [43], and additives [44] which has a significant effect on the analytical performance of ISEs. A second reason can be assigned to interfering ions (lactate and formate), which are present in the wine matrix resulting in higher apparent concentration of acetate in the wine sample using the acetate-selective electrodes for potentiometric measurements. The ionophore used in this thesis (Ionophore 10) has the strongest binding capacity for lactate and acetate out of 11 monocarboxylates, as studied by Martin et al.. It has a similar binding constant as that of ionophore 9 which is regarded as the strongest binder to formate [12].

In order to see any loss of acetate during filtration (as done before IC analysis), the acetate concentration was measured with acetate-selective ISEs in the wine sample with and without filtration procedure. With single addition method without filtration of the wine sample, the acetate concentration was found to be 0.0491 ± 0.0038 M while with filtration it was found to be 0.05392 ± 0.0032 M. However, the concentration of acetate with the double-addition method was 0.0946 ± 0.0003 M for both with and without filtration of the wine sample. This signifies that the filtration procedure does not influence the acetate concentration and is not the reason behind the difference in the concentration measured with IC and the ISEs.

In conclusion, it can be said that the interfering effect from lactate and formate ions in wine and leakage of the ionophore from the ion-selective membrane are the key reasons for inaccurate measurements when the acetate-selective ISEs were used for determination of the acetate content in wine.

5. Conclusions

In this thesis, the suitability of the fabricated solid-contact acetate-selective electrodes for acetate determination in wine was evaluated in comparison with chemically suppressed ion chromatography.

Operational parameters of ion chromatography were successfully optimized by varying the dilution factor of the wine sample, the flow rate and eluent composition to obtain satisfactory peak separation between lactate, acetate and formate. The standard addition method used in ion chromatographic separation corrected the matrix effect on the acetate content in the wine sample.

The galvanostatically synthesized PEDOT(Cl) layer and the ionophore 1,3-bis(carbazolyl)urea derivative incorporated in a polymeric membrane were utilized to fabricate the solid-contact acetate-selective electrodes (acetate-SC-ISEs). With this selective ionophore, low detection limit and high selectivity with near-Nernstian slope were obtained after fresh fabrication and with good piece-to-piece reproducibility. Degraded response of the acetate-selective electrodes with sufficient detection limit and selectivity is assigned to the leakage of selective ionophore from the membrane matrix to aqueous conditioning solution during storage. With the aid of standard addition, which minimizes the matrix effect, acetate determinations were carried out and the potential influence of filtration used in ion chromatography could be excluded.

Leakage of selective membrane components from the acetate-SC-ISEs was observed with lengthened periods of storage in conditioning solution. Therefore, timely experiments should be followed by fresh fabrication of acetate-selective electrodes to obtain excellent detection performance. More studies regarding the lifetime of acetate-SC-ISEs should be carried out to gain insights in the leakage mechanism of the membrane components by means of other analytical methods. If the ionophore is leaking out, then efforts should be made to increase the hydrophobicity of the ionophore. It is also desirable to find new hydrophobic membrane matrix materials substituting the traditional PVC membrane matrix to obtain more robust solid-contact ISEs in potential application in wine production for quality control. In addition, more concerns need to be put on the interfering ions in wine like lactate and formate ions since the ionophore used in this study is also a strong binder to them.

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