APPLICATIONS OF QCM, EIS AND SPR IN THE INVESTIGATION OF SURFACES AND INTERFACES FOR THE DEVELOPMENT OF (BIO)SENSORS [*]

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ABSTRACT

The use of the quartz crystal microbalance, electrochemical impedance spectroscopy and surface plasmon resonance to characterise thin films and to monitor interfaces is presented. The theoretical aspects of QCM, EIS and SPR are discussed and the main application areas are outlined. Future prospects of the combined applications of QCM, EIS and SPR methods in the studies of interfacial processes at surfaces are also discussed.

INTRODUCTION

In the last decades, the analytical sciences have achieved great advances in the possibility to obtain chemical information on objects and systems. As a result, that information has led to developments, in the areas of automation, miniaturization and system simplification[1]. Included are the development and appropriate use of reference systems, qualitative analysis, expansion of classical analytical boundaries (sampling, *in situ* studies, interdisciplinarity) and the development of analytical systems like chemical sensors, biosensors, bioanalytical devices and chemically modified electrodes. In this context, biosensors and chemical sensors have acquired a major importance due to the possible application of these systems to the evaluation of biological or synthetic processes, as well as to the understanding of these processes [2]. Chemical sensors are devices that transform chemical information, like concentration changes of a specific component in a sample in view of the total composition, in a useful analytical signal. These sensors have two basic functional units: a receptor and a transducer [3]. Their application in environmental monitoring and control, in agriculture, food industry, pharmaceutics and medicine has allowed analysis with continuous monitoring in real-time *in vivo* [4].

The development of sensor devices has as basic premise the evaluation of surface and interfacial processes, since these processes are of fundamental importance to the understanding of interaction mechanisms between the receptor and the analyte [5,6]. In addition, the study of surface and interfacial processes is of major relevance for the choice of the species immobilization method on electrode surfaces and the evaluation of problems associated with the immobilization method. Thus, studying these processes can help with the choice of a better immobilization medium and can indicate the major characteristics (advantages and disadvantages) associated with each immobilization method [7-12].

It is worth mentioning the importance that techniques able to evaluate phenomena on surfaces and interfaces have acquired, including spectroscopic, microscopic and/or acoustic methods like attenuated total reflection spectroscopy [13], elipsometry [13], SPR – surface plasmon resonance [14], Raman spectroscopy [15], EIS – electrochemical impedance spectroscopy [16], scanning tunnelling microscopy [17], atomic force microscopy [18], QCM – quartz crystal microbalance [19] and others. However, limitations in the use of techniques like elipsometry, due to its lesser suitability to monitor dynamic processes [20], and

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attenuated total reflection due to its low sensibility for surface processes[21], have directed major attention to QCM [22], EIS [23] and SPR [24]. Electrochemical impedance spectroscopy has acquired major importance, since it allows obtaining a wide range of information from a single experiment [23], as well as complementary information to that obtained by SPR and QCM.

Thus, in this work, the basic principles and individual and combined application of the QCM, EIS and SPR are discussed with the purpose of showing their importance in the investigation of surface and interfacial processes, and highlighting their complementarities.

BASIC PRINCIPLES OF QCM, EIS AND SPR

Quartz crystal microbalance

It consists, basically, of a piezoelectric quartz crystal disc covered by a metallic deposit on the top and bottom side, as shown in Figure 1a. This is, as you can see, coupled to an oscillating circuit that applies an alternating electrical field to the crystal, with the purpose of inducing an oscillation in the centre of the piezoelectric crystal [25]. Directly linked to the oscillating circuit is a frequency counter that monitors the variation in crystal oscillation frequency and a computer for data collection and analysis, as shown in Figure 1b. While the alternating electrical potential is applied, inducing a vibration in the crystal, a transversal acoustic wave is formed that propagates through the crystal. As a result, a displacement of the crystal atoms parallel to the surface occurs. In this way, a reduction in the oscillating movement occurs if a material is deposited on the crystal surface, resulting in a decrease in its resonance frequency. Consequently, by a continuously monitoring the crystal frequency it is possible to study surface and interfacial phenomena (Figure 1c).

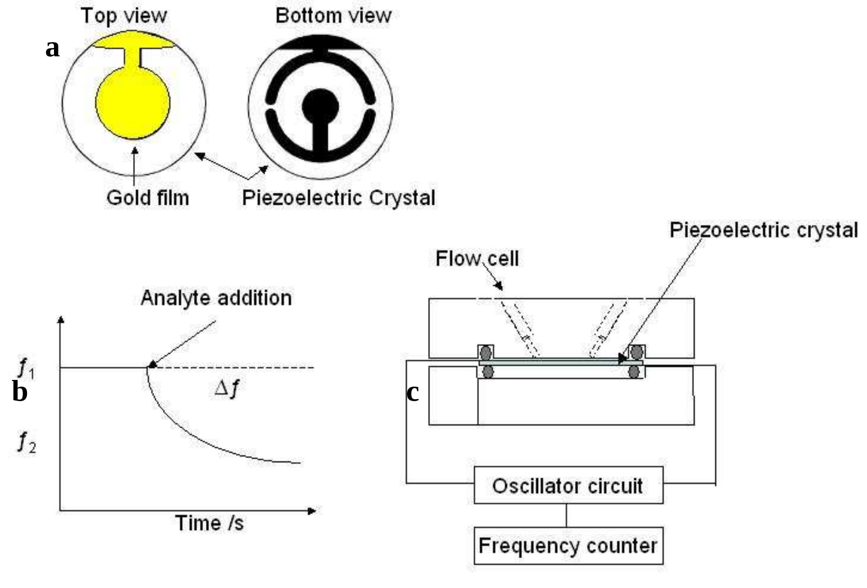


***Figure 1**. (a) Schematic drawing of the quartz crystal top and bottom view with metallic deposits on both sides; (b) scheme of a flow cell for piezoelectric crystals in liquid media, including the oscillating system and frequency counter; (c) schematic massogram representating the variation in quartz crystal frequency as a function of time. (f_1) initial frequency; (f_2) frequency after the addition of analyte; (Δf) frequency change.*

As shown in the pioneering work of Sauerbrey [26], the resonance frequency is a function of crystal mass, such that changes in the system mass, due to species deposition on the crystal surface, can be tracked by following the changes in frequency (Figure 1c) as described by equation [26]

$$\Delta f = \frac{-2\Delta m f_0^{\ 2}}{A\sqrt{\mu_q \rho_q}}$$

where, ρ_q is the quartz density, μ_q - crystal shear module, f_o - crystal fundamental frequency A - crystal piezoelectrically active geometrical area which, is defined by the area of the deposited metallic film on the crystal, Δm and Δf correspond to mass and system frequency changes.

In this context, the theoretical basis developed by Sauerbrey [26] were afterwards applied by King[27] in the development of the first sensor for piezoelectric adsorption of hydrocarbons like o-xylene, etthyl-benzene, n-octane, toluene, n-heptane and n-pentane. The system consisted of a modified piezoelectric quartz crystal (PQC) as detector, and another identical non-modified crystal used as a reference, leading to a new class of fast and selective sensors.

The initial developments by King [27] led to different configurations and interaction mechanisms between acoustic wave and matter, resulting in numerous applications in systems that use the PCQ as mass reader, electroacoustic devices and use the elastic effect. The differences present in these systems led to a wide number of devices based on piezoelectric materials, like “Bulk Acoustic Wave Resonators” (BAW), “Surface Acoustic Wave Resonators” (SAW), “Flexural Plate Wave” (FPW), “Fibre Acoustic Wave” (FAW), “Tube Acoustic Wave” (TAW) and “Transverse Wave Devices” (TWD).

The initial applications of QCM in liquid environments were not successful, since the quartz crystal stopped oscillating when immersed in solution and, the analysis thus were conducted by sample conversion to gas phase or through exhaustive processes of crystal immersion in the solution [28-30].

Konash and Bastiaans [31] conducted the first successful application of an acoustic device as a sensor in liquid phase, , by employing the quartz piezoelectric crystals as mass detectors in liquid chromatography. The development of this system overcame two of the major problems associated with the use of PCQ in liquid environments, which are: stable crystal oscillation and the elimination of effects coming from the liquid viscosity and density.

For the QCM application in liquid systems, factors like liquid density (ρ) and viscosity (η) have to be considered, as the coupling of a crystal surface to a liquid drastically changes its oscillation frequency. This change is caused by the crystal shear movement, which generates a movement in the liquid close to the interface (plano-laminar flux in the liquid) causing a decrease in the crystal oscillation frequency proportional to $(\rho\eta)^{1/2}$ [28], as can be verified from the expression

$$\Delta f = f_0^{\ 3/2}\sqrt{\frac{\rho\eta}{\pi\rho_q\eta_q}} \qquad (1)$$

where ρ_q is the quartz density, μ_q - crystal shear module, f_o – crystal fundamental frequency, ρ the liquid density and η the liquid viscosity.

Nomura and Okuhara [32] studied the use of PCQ in liquids They established an empirical equation that correlates the crystal resonance frequency, the solution viscosity and density for non-conducting liquids, was developed.

After the work by Nomura and Okuhara [32], other research groups proved that, besides viscosity and liquid density, parameters such as the interface solid/solution structure [33], conductivity, polarity and temperature [34-36], interfacial viscosity and crystal surface hydrophilic and hydrophobic characteristics [37-41], film uniformity over the crystal [36] and crystal area in contact with the solution [42] are of major relevance to the application of QCM to liquid environments.

Electrochemical impedance spectroscopy

In Electrochemical impedance spectroscopy a potential or current perturbation is applied to the system under study. The perturbation consists in a DC potential on which a sinusoidal shaped potential signal with small amplitude is superimposed. This method allows the system to be perturbed using only a few milivolts, so that the investigation of electrochemical phenomena close to the equilibrium state is possible. It is furthermore possible to use different frequencies, as the potential signal is sinusoidally shaped. As long as the perturbation on the system under investigation has small amplitude it is possible to employ the technique to analyse the steps of a reactional mechanism [43,44].

In EIS by measuring the relation between applied potential and measured current the system impedance and the phase angle (the phase difference between current and applied potential) are obtained. The concept of impedance, originally introduced to describe the response of a system composed of capacitances, resistances and inductances, was extended to electrochemical systems, where numerous processes contribute to the relation between the current and potential. Thus, based on impedance and phase angle measurements it is possible to evaluate processes like charge transfer (including determination of electron transfer rate), film conductivity, double layer and redox capacitance, charge carriers diffusion coefficients [23], amongst others.

Collecting information from electrochemical impedance data can be achieved by using different measurement models, like equivalent circuits or mathematical models. The application of equivalent circuits is based on the similarity between an electrochemical cell (Figure 2a) and an electrical circuit of resistors, capacitors and inductors.

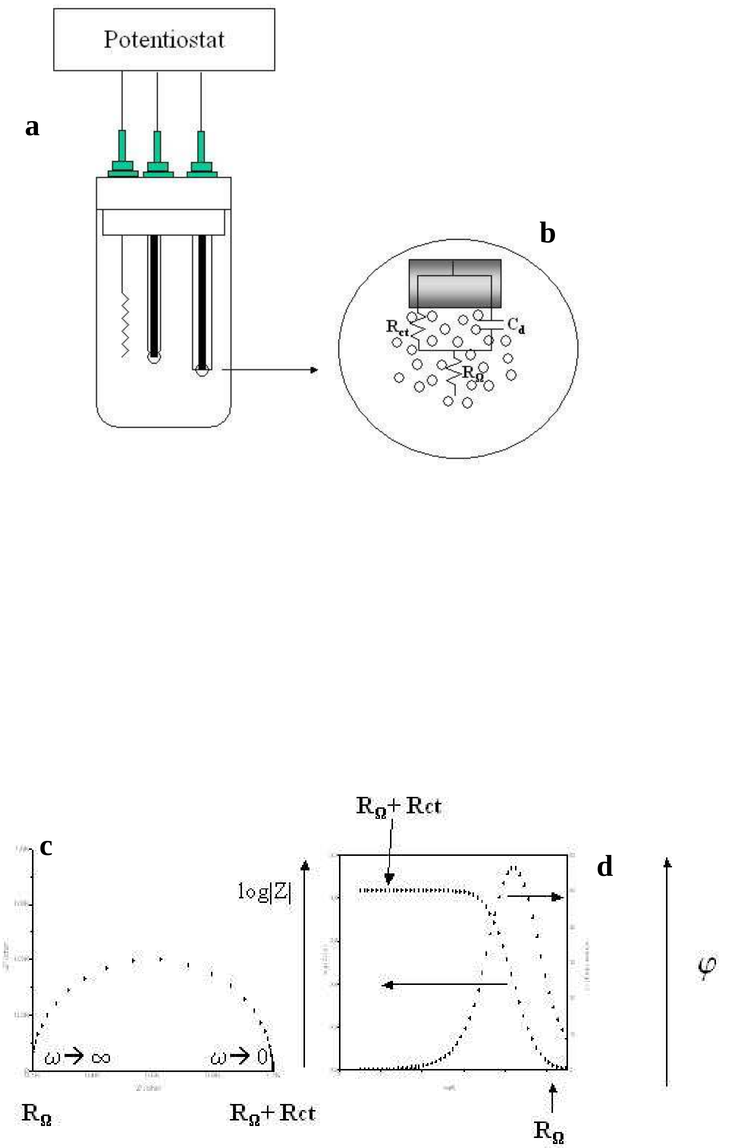


***Figure 2**. (a) Typical three-electrode electroanalytical cell to be used in EIS: (1) counter electrode, (2) reference electrode and (3) working electrode; (b) schematic diagram of a Randles circuit superimposed on the electrode/electrolyte interface; (c) impedance diagram (Nyquist diagram) representing the equivalent circuit shown in the figure; (d) Bode diagram corresponding to the Nyquist diagram.*

A typical description of an electrochemical cell in terms of an equivalent circuit is shown in Figure 2b. The similarity of the electrical double layer to a parallel plate capacitor (Helmholtz model [44]) and the resistance to charge transfer in the electrode/solution interface to a resistance make it possible to represent an interface by a resistor (R_{ct}) and a capacitor (C_d) in parallel. As soon as the current that flows through the electrode/solution interface is conducted by the ions in solution, the resistive effect on the migration of ions in solution is represented by R_W. The difference in phase between current and potential is the result of the introduction of capacitive elements in a circuit. Because of this, a common representation for impedance in systems composed by resistors and capacitors is through a phase diagram in which the impedance shows a real (resistive) and an imaginary (capacitive) component. For the equivalent circuit shown (Figure 2b) the real (Z’) and imaginary (Z”) system impedance components are [44]

$$Z' = R_\Omega + \frac{R_{CT}}{1+\omega^2 {C_d}^2 {R_{CT}}^2}$$

$$Z'' = \frac{\omega {C_d}^2 {R_{CT}}^2}{1+\omega^2 {C_d}^2 {R_{CT}}^2} \qquad \text{(2 and 3)}$$

Where, R_Ω is the solution resistance, R_{ct} is the charge transfer resistance, ω is the angular frequency and C_d is the electrical double layer capacitance.

As can be observed in the expressions above, in the high frequency region the real impedance component tends to R_Ω, while that in the low frequencies region tends to $R_\Omega + R_{ct}$. By evaluating this behaviour it is possible to obtain information on the solution resistance in the high frequencies region and on the electrode processes in the low frequency region (resistance to charge transfer).

On the other side, by eliminating the frequency from the previous expressions a relation between the imaginary impedance and the real impedance is obtained, as shown in the following expression

$$(Z' - R_\Omega - \frac{R_{CT}}{2})^2 + Z''^2 = (\frac{R_{CT}}{2})^2 \qquad (4)$$

It can be verified from the expression above that a graph of Z″ as a function of Z′ results in a (semi-) circular behaviour (Figure 2a) with radius of R_{ct} /2 and the centre in Z′ = R_Ω + R_{ct} /2 and Z″ = 0. In this context, the graphical representation Z″ (imaginary impedance component) *vs*. Z′ (real impedance component), also named Nyquist diagram, Argand, Sluyters or Cole-Cole plot (Figure 2c), can provide information about the possible nature of the elements that contribute to the total system impedance [45,46].

Additionally, log |Z|(|Z| - impedance modulus) and φ (phase angle) *vs*. log ω (ω - frequency) representations, called Bode plots (Figure 2d), are of major importance for the interpretation of data coming from EIS, as the information from these graphs can be complementary to that in the Nyquist diagram. As can be seen in Figure 2d, the changes in the impedance modulus are a measure for the charge transfer resistance. On the other side, as the system frequency is increased, changes in phase angle between applied potential and the resulting current are observed. This change in phase angle gives information on the systems capacitive component, once that the capacitive components introduce a co-sinusoidal behaviour in the current [47].

In this way, the charge transfer resistance (R_{tc}), the electrical double layer capacitance (C_d), as well as the solution effects on ion migration (R_W), can be obtained by the evaluating Nyquist and Bode plots.

Additionally, in systems that show significant impedance effects to mass transfer (Ztm) the introduction of an element denominated “Warburg impedance” is done with the purpose to simulate the experimental system characteristics, for processes that suffer linear diffusion, spherical diffusion or under forced convection. In this context, a number of factors can influence the systems impedance, like faradaic processes, adsorption of electroactive and non-electroactive species, electrode reactions involving the formation of stable intermediates and surface heterogeneities [43], thus generating a wide range of applications field for EIS.

Surface plasmon resonance

Surface Plasmon Waves (SPW) are longitudinal electromagnetic waves, that propagate on the interface between a metal and a dielectric. These waves have been used in the SPR technique due to the fact that they can receive energy from radiation that propagates through the interface. However, in order for the SPW to resonate with the incident radiation, it is necessary that both have equal magnitude wave vectors. If the SPW propagation vector is larger than the one of the incident wave, SPW excitation does not occur by direct contact of these electromagnetical waves. Thus, optical systems were developed to increase the incident wave momentum, such that SPW can receive energy from the incident wave through a resonance energy transfer (Figure 3a). In this way, both the attenuated total reflection phenomenon as well as the diffraction over diffraction grids have been explored [48] by employing the Kretschmann configuration [49] or through optical wave guides [49].

SPR systems that operate by employing the Kretschmann configuration are the ones in wider use, since they generally show higher sensitivity and resolution in relation to the devices that operate by diffraction grids [49,50]. In these systems, during a total internal reflection, the propagation of a fraction of the incident wave on the interface occurs, in order to penetrate in the medium of lower optical density resulting in an evanescent electromagnetic field (Figure 3a).

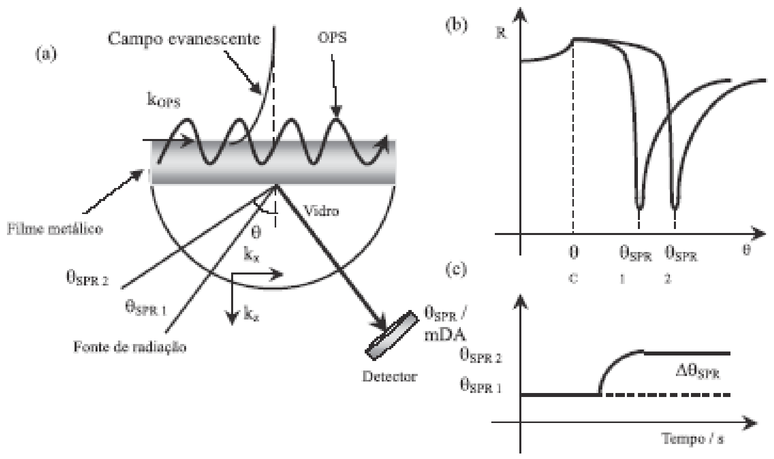


***Figure 3**. (a) SPR Kretschmann configuration schematic representation. The dielectric in contact with the metal allows energy transfer from the incident wave to SPW; (b) reflectance plots in absence (θ_{SPR1}) and presence (θ_{SPR2}) of species on the metallic film surface; (c) schematic sensorgram representating the relation between SPR angle (θ_{SPR}) and time during the interaction of species with the metallic film surface.*

While stretching into environment this fraction of the incident radiation couples with the metal oscillating free electrons, such that, changes in the vicinity of the metal/environment interface promote a change in the system resonance conditions. As a result, a displacement in the SPR angle occurs (Figure 3b). As can be verified in the following equation, the parallel component of the incident light has the vector wave (k_x) related to the light angle of incidence.

$$k_x = \frac{\omega}{c}\sqrt{\varepsilon_p \sin\Theta} \qquad (5)$$

Where Θ it is the incidence angle with the metal surface, ε_p the prism dielectric constant, ω the angular frequency of the incident light and *c* the speed of light. Additionally, the SPW propagation constant (k_{SPW}) in a metal/dielectrical interface is expressed by:

$$k_{SPW} = \frac{\omega}{c}\sqrt{\frac{\varepsilon_m \varepsilon_p}{(\varepsilon_m + \varepsilon_p)}} \qquad (6)$$

Where ω is the wave angular frequency, *c* is the speed of light, ε_p is the dielectric constant of the species that interacts with the surface and ε_m is the metal dielectric constant. Thus, it is possible to use the angle of incidence as a control parameter for the SPW phenomenon, by monitoring reflectance *vs*. angle of incidence (Figure 3b). As soon as the parallel component of the incident light is equivalent to the SPW propagation constant, *i.e.*, $k_x = k_{SPW}$ (Equations 5 and 6) a drop in reflectance will be found, due to the formation of an evanescent wave, that propagates through the metallic surface and interacts with the external medium, as can be verified by equations 7 and 8.

$$\frac{\omega}{c}\sqrt{\varepsilon_p} \sin\Theta = \frac{\omega}{c}\sqrt{\frac{\varepsilon_m \varepsilon_p}{(\varepsilon_m + \varepsilon_p)}} \qquad (7)$$

$$\Theta = \arcsin\left(\sqrt{\left(\frac{\varepsilon_m \varepsilon_p}{(\varepsilon_m + \varepsilon_p)} x \frac{1}{\varepsilon_p}\right)}\right) \qquad (8)$$

In this last expression it can be seen that the system optical properties, like the metal, prism and matrix dielectric constants, induce changes in the resonance angle, making it possible to apply the SPR phenomenon to monitor changes in the sensor surface, by plotting the resonance angle vs. time (Figure 3c). Thus, by monitoring the refractive index in the vicinity of the sensor disc surface it is possible to use the SPR to obtain information on the velocity and the amount of adsorption, allowing the determination of dielectric properties, association and dissociation kinetics, as well as affinity constants for specific interactions [24].

From the theory of each technique it is possible to evaluate its fields of application, as well as to establish its possibilities and limitations. In this context, by checking the theory associated with QCM and SPR it can be observed that acoustic waves and surface plasmon waves are not phenomena where loss of energy occurs by means of radiation, but by means of evanescent waves. Thus, it is possible to establish theoretical limits to the penetration depth of these evanescent fields, as can be seen in the following expressions [22]

$$\alpha_{QCM} = \sqrt{\frac{2\eta}{\omega\rho}}$$

$$\alpha_{SPR} = \frac{\lambda}{2\pi\sqrt{\varepsilon}} \qquad \text{(9 and 10)}$$

Where α_{QCM} and α_{SPR} are the penetration depth of the evanescent waves generated in QCM and SPR. η and ρ are, respectively, the viscosity and the density of the liquid in which the crystal is immersed, ω is the crystal angular excitation frequency, λ is the incident wave length, and ε the liquid dielectric constant. EIS does not suffer from the same limitations as it is based on the perturbation of the system by application of a small potential or current signal.

SURFACE AND INTERFACIAL PROCESSES AND ANALYTICAL APPLICATIONS OF QCM, EIS AND SPR

The theory developed for the application of the quartz crystal microbalance in liquid environments, allows its use in the characterization of biomolecular systems, with the purpose of sensor development, as well as the investigation of interfacial processes. [51,52]. Okahata and co-workers [53] carried out kinetic measurements of DNA hybridization on piezoelectric quartz crystals with 27 MHz of fundamental frequency, modified with complementary oligonucleotides through avidin-biotin bridges.

Based on this, a wide range of research on hybridization between oligonucleotides and possible damaging agents to deoxyribonucleic acid has been published[54]. Cosnier and co-workers [55] used a quartz crystal microbalance to carry out an investigation of the microgravimetric processes of the electropolymerization of polypirrol-biotin films, with the goal to produce matrices with high reproducibility in order to be able to deposit biological macromolecules like glycose oxidase.

The applicability of piezoelectric quartz crystals is extended to research of charge and mass transfer processes in metalopolymers, since these phenomena directly influence parameters, like the apparent charge transfer coefficient, that are of fundamental importance to the interpretation of mass and charge transfer phenomena in polymeric films [56,57]. In addition, the quartz crystal microbalance makes it possible to separate charge and mass transfer phenomena, in order to contribute, fundamentally, to the investigation of interfacial phenomena [58].

In this way, the quartz crystal microbalance allows an isolated study of interfacial phenomena, to distinguish between different types of interfacial reactions, allowing the classification of the possible reactions. Fletcher and co-workers [58] used a quartz crystal microbalance to generate massograms that, together with voltammetric data, generated a classification of the interfacial reactions. These studies lead to the classification of interfacial reactions in, basically, five types: reactions where faradaic processes occur and that are associated to mass changes, like electrodeposition, electrodissolution and intercalation reactions; reactions where faradaic processes occur that are not associated to mass changes, like gas evolution reactions; non-faradaic processes that are not associated with mass changes, like capacitive processes; non-faradaic processes associated with mass changes, like specific adsorption processes of perchlorate ions over gold surface and processes where mass changes occur, but which are not associated to faradaic or non-faradaic processes. Some examples of interfacial processes studies, related to the development of piezoelectric sensors, are shown briefly, in Table 1.

Table 1. Examples of applications of the quartz crystal microbalance for the investigation of surface and interfacial processes.

Application	Goal	Ref.
Organic films	• use of a BAW device for evaluation of solvent and film thickness effects in the film formation process	92
	• study by QCM of the parameters associated with adsorption, as well as identification and division between chemical and physical adsorption during the film formation stage	93
Langmuir–Blodgett films	• monitoring of copper (II) 1,4,8,11,15,18,22,25-octabutoxiftalocianine films thickness	94
	• study of liposome adsorption and desorption in solutions and emulsions	95
Self-assembled monolayers	• study of globotriaosyl ceramide (Gb3) interaction with shiga toxin 1 and 2	96
	• study of butanethiol formation kinetics on gold	97
Protein films	• adsorption kinetics and viscoelastic properties of Protein A (coming from *Staphylococcus aureus*), bovine serum albumin, Immunoglobulin G and fibronectin	98
	• study of interaction (in real time) and association, dissociation and equilibrium constants determination of berberine chloride with bovine serum albumin	99
Polymers conducting	• evaluation of redox processes and conductivity mechanisms of polypyrol films modified with different anions	100
	• investigation of mass transport processes and monitoring of diphenylamine electropolymerization process	101
	• study of mass changes and redox processes during aniline electropolymerization in presence of sodium dodecilsulphate	102
Supramolecular systems	• study of enantioselective interaction of calix[4]arenes films with (R) methyl-lactate and (S) methyl-lactate	103
	• adsorption and desorption kinetics of toluene and chloroform vapours over t-butyl-calix[6]arene films	104

Besides application as a tool for research of interaction phenomena with the purpose of sensor development, piezoelectric quartz crystals have intensively been applied in adsorption kinetic studies in several matrices. In this way, protein adsorption kinetics [59], alkanetiols [60], surfactant species like sodium dodecilsulfate [61], metals like copper [62], among others, have been investigated. These studies are possible due to the ability of the technique to simultaneously measure frequency change and energy dissipation of the piezoelectric quartz crystal, during the adsorption process [63].

By monitoring the crystal resonance frequency it is possible to determine mass changes and, consequently, to study different interfacial processes in thin films, to determine species transport, adsorption kinetics and growth.

In this research context of surface and interfacial phenomena, electrochemical impedance spectroscopy has been widely applied, as a complementary technique, to several fields of electrochemistry, like electrode kinetics [64-66], double layer studies [67], battery processes [68], investigation of corrosion processes [69,70], solid state electrochemistry [71] and bioelectrochemistry [72], especially to processes in membranes.

By employing EIS, Heiduschka and co-workers [73] developed mixed self-assembled monolayers of ω-undecanotiol over gold modified with viral antigen fragments (epitopes), with the purpose of reducing the need of separation methods between antigen and antibody, thus minimizing the damage to the receptor. Once the monolayers were formed, the impedance measurements were used to measure the capacitive component, to continuously monitor the interaction between antigen and antibody. In this context, Farace and co-workers [74] applied EIS to the development of a sensor system that operates through affinity, like antigen – antibody interactions and interactions between oligonucleotides complementary chains. Ouerghi and co-workers [75] applied EIS in a system containing poli-2-cyan-ethylpyrrole modified with rabbit polyclonal antibody as a sensor to the rabbit polyclonal antigen. The EIS was applied to the investigation of the increase in polymer resistance, which is related to the increase of the resistance to charge transfer, as well as to measure decrease in the system diffusional impedance, as it allows obtaining relevant information about optimum operation conditions the working electrode.

With the purpose of establishing better film formation conditions [76], an EIS application has been reported to study surface coverage of calix[4]arene deposits. EIS has also been used to study film uniformity in multilayers [77,78], in order to establish the best conditions to form compact resistant films with a high sensitivity for the analyte.

EIS has also been applied in research of charge transfer mechanisms in films, like antibody or avidine modified polypyrrole by evaluating the impedance behaviour as a function of excitation frequency. The goal of these studies is to indicate the relation between changes in the polymer and the interactions resulting from bioaffinity between receptor and analyte[79]. In this context, Kasen [80] applied EIS to evaluate diffusion and kinetic concomitant processes in glassy carbon electrodes modified with ferro-hexacyanorutenate (II) films, by evaluating kinetic constants of different parameters related to diffusion, charge transfer and double layer capacitance.

The application of EIS in the analytical field has been extended to the study of DNA oligomers hybridization, by following the total impedance. These investigations are possible as the total impedance is associated to the increase of the capacitive component. Furthermore, the system capacitive component is a result of density and ion mobility changes associated with the hybridization reaction [81]. Vetterl and co-workers [82], with the purpose to evaluate differences between double-helix conformation and simple conformation, as well as the adsorption kinetics and adsorbed polynucleotide segments mobility, studied the double layer capacitance in electrodes containing adsorbed DNA.

The application of EIS has been extended to the investigation of conducting properties from polymers like polyacetylene, polypyrrole, polytiophene, polyaniline, amongst others. Munichandraiah and Prasad [83] evaluated the catalytic activity of polyaniline modified platinum electrodes for the oxidation of Fe^{2+} and hydroquinone. By determining the system's catalytic efficiency, from equilibrium current and intrinsic velocity constant, these studies led to optimized applications of these polymers in corrosion protection systems, batteries, catalysts in oxidation reactions, amongst others [84]. Table 2 shows, briefly, some EIS applications related to the development of impedimetric biosensors.

Table 2. Examples of the application of electrochemical impedance spectroscopy in the research of surface and interfacial processes.

Application	Goal	Ref.
Organic films	• Determination of acid-base equilibrium constants (pK) to mercapto propionic acid, mercapto hexadecanoic acid and mercaptododecil amine monolayers	105
Langmuir–Blodgett films	• Determination of electron transfer processes, including resistance to charge transfer and faradaic capacitance in poly(N-dodecil acrylamide)-co-(4(acrylomethyl)4'-methyl-2,2'-bipiridine)-bis(2,2'-bipiridine) ruthenium diperchlorate polymeric films	106
	• to study mass and charge transfer processes of dimethyldioctadecil ammonium and Prussian blue hybrid films	107
Self-assembled monolayers	• to study the adsorption kinetics and degree of coverage for octanethiol, decanethol, dodecanethiol, hexadecanethiol and octadecanethiol SAMs	108
	• characterization defects and coverage degree in octadecanethiol self-assembled monolayers on gold surfaces	109
	• obtain the charge transfer rate coefficient in glutation monolayers on gold	110
Protein films	• adsorption kinetics of horse heart cyitochrome *c* over carboxythiol monolayers on gold surfaces	111
	• influence of β-lactoglobulin adsorption process on stainless steel over the metallic material corrosion kinetics and mechanism	112
Conducting polymers	• determination of the capacitive behaviour of a polypyrrole/polyimide composite	113
	• to study mechanisms of ion and electronic conductivity, and characteristics of ion exchange in polyaniline films	114
Supramoleculars systems	• evaluation of electron transfer kinetics in self-assembled monolayers of inclusion complexes between α-, or β-cyclodextrine and N-(n-octyl)-N'-(10-mercaptodecyl)-4,4'-bipyridine bromide	115

On the other side, due to great interest in the investigation of real time surface processes, the first application of SPR systems by Liedberg and co-workers was conducted in 1983 [85]. By adsorption studies of γ-globulin antigen over a silver film surface and later addition of γ-globulin antibody in different concentrations, it was possible to show that SPR can be applied in an extremely sensitive and selective way. In this way, a growing interest in specific interaction studies between biomolecules has been developed, with the purpose to investigate the affinity and the intermolecular interaction kinetics.

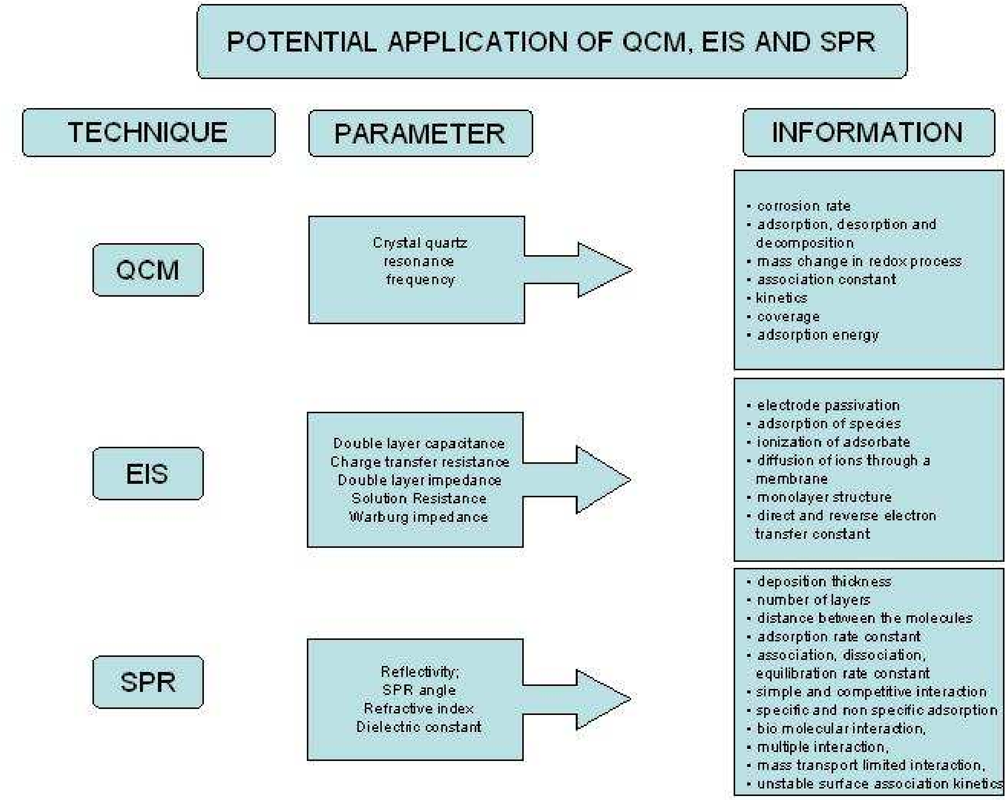
The biospecific interaction analysis in real time through SPR has acquired growing interest due to the information it can provide about biomolecule properties, like interaction models, intermolecular affinity and kinetic constants [86]. With the purpose of investigating optical properties by SPR, two configurations have been applied to the study of the interface: (1) detection by direct biomolecular interaction over a metal surface and (2) detection by interaction in hydrogel matrix. One of the molecules is immobilized on the sensor surface or matrix, so that interactions with other molecules induce shifts in the SPR resonance angle, that are directly related to the amount of molecules that interact with the sensor surface.

The use of a hydrogel matrix makes it possible to covalently bond one of the molecules so that a higher number of specific interaction sites per surface area become available. Furthermore, biomolecule immobilization ability in a matrix is higher and, thus the use of a matrix will increase the system sensitivity [87] and improve the molecule accessibility [88]. Through this SPR allows the study of a biomolecular interaction in real time without the use of labelling reagents, so that such a system can be used to determine molecule concentration, and affinity and interaction kinetics [89]. Table 3 shows some SPR applications related to the development of sensor devices.

Table 3. Examples of the application of surface plasmon resonance in the investigation of surface and interfacial processes.

Application	Goal	Ref.
Organic films	• to evaluate the thickness, porosity and structure of azobenzene films	116
	• to study the thickness, dielectric constant and conductivity of 3(ω-mercaptoundecyl) modified polypyrrole films	117
Langmuir–Blodgett films	• to calculate the thickness, refraction index and extinction coefficient of calix[4]resorcinarene films	118
Self-assembled monolayers	• to study the degree of coverage and, thickness of mercapto-hexanol modified DNA films, as well as adsorption and desorption kinetics	119
	• to evaluate the formation kinetics of poly(γ-benzyl L-glutamate) self-assembled monolayers on gold	120
	• to study the interaction of acid dithiobis(4-butyrilaminom-phenylboron)monolayers with fructose, mannose and galactose	121
Proteic films	• to study the interaction of bacteria *Bacillus thuringiensis* proteins (σ-endotoxins), rate constants for association, dissociation and affinity constants	122
Conducting polymers	• to evaluate the relation between polyaniline film thickness on gold and number of potential cycles, as well as conductivity, dielectric constants of the film in oxidised and reduced form and film formation kinetics	123
	• to study the adsorption kinetics of organic molecules like toluene, propane and butane over calix[4]resorcinolarene films and adsorption influence on film refraction index and thickness	124
Supramolecular systems	• to study the interaction kinetics between tetra-undecyl-tetra-p nitrophfenylazocalix[4]resorcinarene and tetra-undecyl-tetra-(4-aminomethyl-4’-nitroazobenzyl)calix[4]resorcinarene films with benzene	125

The analytical possibilities of electrochemical impedance spectroscopy, surface plasmon resonance and quartz crystal microbalance, as well as the importance of its applications to the research of surface and interfacial processes are closely linked to the development of trusted methods. In this context, the combined use of these techniques has been explored with the purpose of providing complementary information that allows secure interpretations of the systems under evaluation (Scheme 1).



Scheme 1. *Major parameters and information obtained in QCM, EIS and SPR applications.*

With this purpose, Vogel and co-workers [90] used impedance spectroscopy and surface plasmon resonance simultaneously in the investigation of interactions between cholera toxin and ganglioside. In order to generate quantitative information on lipids in solid support, SPR measurements were done. EIS was employed to measure receptor content in the lipid layer. Offennhaussen and co-workers[91] studied properties of self-assembled monolayers of alkylthiols and thiolipids on gold. Using SPR and EIS the monolayer average thickness and the electron transfer and adsorption kinetics were determined. Recently QCM and EIS were applied together by Guiseppe-Elie and co-workers [81] in studies of DNA hybridization, by measuring impedance and frequency changes associated with oligonucleotide conformational changes.

With the purpose of reducing the interferences resulting from the combination of QCM and EIS, Yao and co-workers [92] developed a new strategy to simultaneously apply quartz crystals and electrochemical impedance (impedance in piezoelectric quartz crystal - IPCQ). This hybrid system was applied in the investigation of bovine albumin adsorption on gold and platinum electrodes, showing that IPCQ allows on the study of electrode mass, film viscoelasticity, solution local viscosity/density close to the electrode surface and interfacial capacitance. Table 4 shows some combined techniques, highlighting their individual possibilities.

Table 4. Examples of combined SPR, QCM and EIS applications for the study of surface and interfacial processes.

Application	Goal	Ref.
EIS and QCM	• EIS used in the study of the relationship between ion movement and polarizable molecules in DNA hybridization and QCM applied to distinguish between complementary and non-complementary hybridization processes	81
	• QCM used to monitor in real-time the protamin adsorption followed by heparin adsorption, as well as optimisation of interaction processes (protamin-heparin) and determination of viscosity and heparin solution density effects on the analytical signal. EIS was applied to determine the physical parameters: solution resistance, resistance to charge transfer, electrical double layer capacitance and its relations with the heparin concentration	72
	• EIS used to determine capacitive behaviour of polyanaline films modified with Molybdenum trisulphate and QCM to monitor the mass transport in the composite, to elaborate cation, anion and neutral species transport mechanisms in the film	126
SPR and EIS	• SPR to monitor in real-time avidin-biotin multilayer formation on gold and determination of the surface molecule concentration and EIS, to study the relation between layer composition and resistance to charge transfer and double layer capacitance	127
	• EIS to study species adsorption and desorption on conducting materials and its influence on the electron interfacial transfer and SPR used to monitor in real-time the lipid bilayer formation, and to determine surface lipid density	127
QCM and SPR	• SPR used to measure protein adsorption kinetics modified with endoglucanase and QCM to measure adsorbed protein layer rigidness	128
SPR, QCM and EIS	• SPR applied to determine film thickness during the growth process, providing information on the monolayer formation dynamics; the QCM information allowed a differentiation between di-phtanoilphosphatedilcoline vesicles adsorption and fusion on the self-assembled monolayers, while EIS allowed the monitoring of the film physical parameters	129

CONCLUSIONS AND FUTURE PERSPECTIVES

This work has discussed some potential applications of electrochemical impedance spectroscopy, surface plasmon resonance and quartz crystal microbalance, focusing on surface and interfacial phenomena and showing how these techniques can help in building sensor devices. The importance of the combined use of these techniques is based on the research features attributed to each one of them. Both QCM and SPR can provide measurements on the dynamics of real time events, the QCM being able to provide information on the system mass variations, leading to important information on species transfer in films. SPR can complement these measurements on the process dynamics based on a high sensitivity level and EIS can generate information on the conducting properties of the developed systems.

In this way, the combined application of EIS, SPR and QCM is a powerful tool to study a wider number of properties related to the surface and interfacial processes leading to a higher reliability of the research results. These techniques are promising tools for *in situ* application, individually, in a combined form or even simultaneously. Consequently, their application to development of films acting as support of/or receptors in systems sensors is fundamental, because they provide the possibility to obtain detailed knowledge of important properties of the systems under study.

As a future perspective, a great development and application of QCM and SPR, combined with the electrochemical techniques (fundamentally EIS) is expected, in order to generate a wide and informative data group, simultaneously leading to detailed measurements of surface and interfacial systems contributing, in a decisive manner, to the understanding of these processes as well as to the strengthening and expansion of the EQCM and ESPR applications.

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