ESCUELA TÉCNICA SUPERIOR DE INGENIEROS
DE TELECOMUNICACIÓN

UNIVERSIDAD POLITÉCNICA DE MADRID

DISEÑO Y EVALUACIÓN DE UN BIOSENSOR
BASADO EN RESONADORES DE CRISTAL DE
CUARZO (QCR) PARA CARACTERIZAR
MUESTRAS BIOLÓGICAS RELACIONADAS
CON ENFERMEDADES ARTRÍTICAS

TESIS DOCTORAL
DOCTORADO EN INGENIERÍA BIOMÉDICA

Luis Armando Carvajal Ahumada
Ingeniero en Electrónica

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DEPARTAMENTO DE TECNOLOGÍA FOTÓNICA Y
BIOINGENERÍA

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Luis Armando Carvajal Ahumada

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José Javier Serrano Olmedo

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Oscar Leonardo Herrera Sandoval

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AUTOR: Luis Armando Carvajal Ahumada

DIRECTORES: José Javier Serrano Olmedo, Oscar Leonardo
Herrera Sandoval

Tribunal nombrado por el Magfco. y Excmo. Sr. Rector de la Universidad
Politécnica de Madrid el 28 de marzo de 2017.

PRESIDENTE: Dr. D. Francisco del Pozo

SECRETARIO: Dr. D. Miguel Holgado

VOCAL: Dr. D. Tomas Gómez

VOCAL: Dr. Dña. Cristina Sánchez

VOCAL: Dr. D. Nazario Félix

SUPLENTE: Dr. D. Miguel Manso

SUPLENTE: Dr. D. Javier Buldú

Realizado el acto de defensa y lectura de tesis el día 03 de abril de 2017 en
el Centro de Tecnología Biomédica de la Universidad Politécnica de
Madrid.

Calificación:

EL PRESIDENTE:

EL SECRETARIO:

Dr. D. Francisco del Pozo

Dr. D. Miguel Holgado

EL VOCAL:

EL VOCAL:

Dr. D. Tomas Gómez

Dr. Dña. Cristina Sánchez

EL VOCAL:

Dr. D. Nazario Félix

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Resumen:

Los resonadores de cristal de cuarzo son dispositivos ampliamente utilizados como biosensores debido a su alta sensibilidad ante fenómenos físico-químicos que ocurren en su superficie, a la reproducibilidad de las mediciones y por ser transductores fáciles de utilizar. Sauerbrey[1] fue el primero en demostrar que el resonador cristal de cuarzo puede ser utilizado como una microbalanza útil para caracterizar pequeñas deposiciones de masa en el electrodo del cristal. Sauerbrey demostró que, si se deposita una delgada capa de material rígido sobre el cristal de cuarzo, tanto el cristal como el material depositado vibrarán a la misma frecuencia y se generará un cambio en la frecuencia de resonancia inicial del cristal. Específicamente, la frecuencia de resonancia disminuye al aumentar la cantidad de masa depositada.

El cristal de cuarzo también puede operar en medio fluidos. En este caso además del desplazamiento en frecuencia del cristal, se genera una pérdida significativa en el factor de calidad del resonador. Para fluidos Newtonianos (viscosidad constante), Kanazawa y Gordon[2] demostraron que el desplazamiento en frecuencia que sufre el resonador así como el cambio en el ancho de la curva de admitancia, depende de la densidad y la viscosidad del líquido. Este fenómeno permite utilizar los resonadores de cristal de cuarzo como pequeños viscosímetros siempre que se conozca la densidad del fluido a analizar.

En el caso de deposición de fluidos viscoelásticos, la medición del cambio en la frecuencia de resonancia del cristal no es suficiente para obtener el valor de viscosidad del fluido. Por tanto, se requiere de la medición de una segunda variable (mitad del ancho de media banda - Γ) para obtener el valor correcto de la viscosidad del fluido depositado.[3] Para fluidos pseudoplásticos (viscosidad aparente dependiente de la velocidad de cizallamiento aplicada), se requiere de un modelo matemático que explique el comportamiento de la viscosidad del fluido a distintas velocidades de cizallamiento. En particular para el análisis de muestras con este comportamiento, se ha elegido el modelo Rouse ya que explica con suficiente detalle el comportamiento pseudoplástico y resulta simple de aplicar a las medidas experimentales.

En este trabajo se propone el diseño de un biosensor basado en un resonador de cristal de cuarzo (QCR) para la caracterización de muestras biológicas que emulan el comportamiento del líquido sinovial.

El líquido sinovial es una mezcla de plasma dializado y ácido hialurónico con comportamiento pseudoplástico. Su principal función es la de actuar como lubricante en las articulaciones

manteniendo al mínimo la fricción entre los huesos, especialmente durante el movimiento. [4–7] La disminución del ácido hialurónico en el líquido sinovial se ha relacionado con enfermedades artríticas ya que las propiedades lubricantes del líquido sinovial dependen de su viscosidad y, al mismo tiempo, esta viscosidad depende de la concentración de ácido hialurónico en el fluido sinovial. [4–8] Está ampliamente documentada la relación que existe entre los cambios de viscosidad del líquido sinovial y las afecciones articulares.[4–13] Con base en la relación existente entre la concentración de ácido hialurónico y las enfermedades artríticas, el análisis del líquido sinovial puede aportar información fundamental para el diagnóstico y tratamiento de estas alteraciones en las articulaciones.

Con base en lo anterior, la aplicación biomédica propuesta tiene como finalidad aportar a la diagnosis de enfermedades artríticas tal como la osteoartritis y artritis reumatoide analizando la viscosidad del fluido sinovial. Debido a que este es un trabajo preliminar, se utilizan muestras que emulan el comportamiento del fluido sinovial y no muestras clínicas.

DESIGN AND EVALUATION OF A BIOSENSOR BASED ON QUARTZ CRYSTAL RESONATOR (QCR) TO CHARACTERIZE BIOLOGICAL SAMPLES RELATED TO DIAGNOSIS OF ARTHRITIC DISEASES

Quartz crystal resonator is widely used as biosensor device due to its high sensitivity to physicochemical phenomena occurring on its electrode surface, the reproducibility of its measures and its easy use. Sauerbrey[1] was the first to show that the quartz crystal resonator can be used as a microbalance useful for characterizing small layers of mass deposited on the crystal electrode. Sauerbrey observed that if a thin layer of rigid material is deposited on the quartz crystal, the crystal and the deposited material will vibrate at the same frequency generating a change in the initial resonance frequency of the crystal. Specifically, the resonance frequency decreases when increases the deposited mass.

The quartz crystal can also operate in fluid medium. In this case, in addition to the frequency shift of the crystal, there is a significant loss in the quality factor of the resonator. For Newtonian fluids (constant viscosity), Kanazawa and Gordon [2] note that the frequency shift and the change in the quality factor of the crystal, depends on the density and viscosity of the liquid deposited. This phenomenon allows to use quartz crystal resonators as small viscometers. The density of the fluid to be analyzed must be known.

For deposition of viscoelastic fluids, the measure of resonance frequency shift is not sufficient to obtain the fluid viscosity value. Therefore, it is necessary to measure a second variable (the half band half width - Γ) to obtain the correct value of viscosity of the deposited fluid [3]. For pseudoplastic fluids (apparent viscosity dependent of the shear rate applied), it is necessary a mathematical model to explain the behavior of the fluid viscosity at different shear rates. In particular, for the analysis of samples with pseudoplastic behavior, Rouse model has been chosen because it explains in sufficient detail the pseudoplastic behavior and is simple to apply to the experimental measurements.

In this work, the design of a biosensor based on a quartz crystal resonator (QCR) for viscometric characterization of biological samples emulating the behavior of the synovial fluid is proposed.

Synovial fluid is a mixture of plasma and hyaluronic acid dialysate with pseudoplastic behavior. Its main function is to act as a lubricant in joints keeping to a minimum the friction between the bones, especially during movement [4–7].

The decrease of the hyaluronic acid in the synovial fluid has been associated with arthritic disease since the lubricating properties of synovial fluid depend on its viscosity and the same time, this viscosity depends on the concentration of hyaluronic acid in the sample. [4–8] It is widely documented the relationship between changes in viscosity of the synovial fluid and joint

conditions [4–13]. Based on this relationship between hyaluronic acid and some arthritic diseases, the synovial fluid analysis can contribute fundamental information to diagnosis and treatments for joint diseases.

Based on the above, the biomedical application proposed is intended to provide the QCR's as an additional tool for the diagnosis of arthritic diseases such as osteoarthritis and rheumatoid arthritis analyzing synovial fluid viscosity. Because this is a preliminary work, hyaluronic acid dilutions are used in order to emulate the behavior of synovial fluid.

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INDEX

1. INTRODUCTION.....	1
2. PRESENT PROBLEMATIC AND HYPOTHESIS	3
3. GOALS	4
4. CHAPTER 1 - Fundaments and QCR Theory	5
4.1. Equivalent electrical model BVD.....	7
4.2. QCR in contact with fluids.....	11
4.3. Newtonian Fluids	13
4.4. Non-Newtonian Fluids	13
4.5. Rouse Model	15
4.5.1. Constitutive Equations	16
4.6. Hyaluronic Acid	17
4.7. Colloidal Dispersions	20
4.8. Steps for characterizing the Quartz Sensor	21
4.9. Acquisition systems for piezoelectric sensors.....	22
4.9.1. Impedance analyzer.....	22
4.9.2. Adapted equipment of sweep frequency	23
4.9.2.1. The Calvo circuit.....	23
4.9.3. Impulse excitation and decay method	24
4.9.4. Oscillators.....	25
4.9.5. Lock-In Techniques.....	26
5. Multichannel Quartz Crystal Resonators	27
6. Chapter II – The Biosensor Design	30
6.1. Crystals and Holders	30
6.2. Proposed Design.....	32
6.3. Simulations.....	33
6.3.1. Geometric Model.....	33
6.3.2. Equivalent Electric Model (BVD).....	35
6.4. Simulations in Matlab	42
6.5. First Version of the Biosensor.....	44

6.5.1.	Stage 1 – Acquisition Data.....	44
6.5.2.	Stage 2 – Processing Data	46
6.5.3.	Relative Permittivity measure with QCR.....	49
6.6.	Second Version of Biosensor	50
6.6.1.	Improve Acquisition Data	50
6.6.2.	Improve Processing Data	51
6.6.3.	Improve Supply System	53
6.7.	Final Version of Biosensor.....	54
6.7.1.	Improved User Interface.....	55
6.7.2.	Multichannel quartz crystal	57
6.7.3.	Auto-Gain Adjustment System	58
6.7.4.	Protocol of measure.....	59
6.7.5.	Protocol to clean crystals.....	59
6.7.6.	Protocol to obtain the Biosensor Performance	60
6.8.	Results – Experimentation	60
6.9.	Sensibility and Limit of Detection of the Biosensor	64
6.10.	Results – Relative Permittivity measure with QCR	67
6.11.	Discussion – Equivalent electric model proposed with Cs	68
7.	Chapter III – The Proposed Biomedical Application: Potential diagnosis of arthritic disease	69
7.1.	Introduction	69
7.2.	Elaboration of the Samples	71
7.3.	Results – Egg white samples.....	71
7.4.	Results – Hyaluronic acid samples.....	73
7.5.	Discussion	76
7.5.1.	Additional Considerations	76
8.	CONCLUSIONS.....	78
9.	FUTURE WORK	79
	REFERENCES.....	80
	APPENDIX A: Maxwell Constitutive Equations	89

FIGURE INDEX

Figure 1. Frequency response of the crystal admittance	5
Figure 2. Increase of the crystal thickness due to the mass deposition. (reproduced from [3])	6
Figure 3. Butterworth-Van-Dyle equivalent circuit for undamped crystal. (adapted from [37]) ..	7
Figure 4. Butterworth-Van-Dyle equivalent circuit for damped crystal. (adapted from [37])	8
Figure 5. Locus diagram of the crystal admittance. (Adapted from [56])	11
Figure 6. Basic concepts for viscosity theory (reproduced from [59])	12
Figure 7. Typical flow curves for Newtonian, shear thinning and shear thickening (dilatant) fluids: (a) shear stress as a function of shear rate; (b) viscosity as a function of shear rate (reproduced from [61])	14
Figure 8. Different molecule conformations in polymer dilutions	14
Figure 9. Viscosity behavior according the molecule conformation. (adapted from [59])	15
Figure 10. Polymer chain conformation according to Rouse Model (reproduced from [70])	16
Figure 11. Chemical Structure of HA (reproduced from [74])	17
Figure 12. Physical conformation of HA, radius of gyration. (reproduced from [76])	18
Figure 13. Fungilab Smart Viscometer	19
Figure 14. Profile for the Zero Shear Viscosity of a HA dilution with low viscosity	20
Figure 15. Precision Impedance Analyzer (reproduced from www.keysight.com/)	22
Figure 16. Sensor schematic for Calvo technique (Adapted from [81])	23
Figure 17. shows the resultant spectrum function $ V_o/V_i $ (Reproduced from [81])	23
Figure 18. Impulse excitation QCM-D method. (Adapted from [90])	25
Figure 19. Circuit block diagram of Nakamoto (Adapted from [42])	26
Figure 20. Circuit block diagram of Nakamoto (Adapted from [42])	27
Figure 21. Sensor schematic for Jakoby technique (Adapted from [47])	27
Figure 22. mesa-shaped structures: (a) plano-mesa (b) plano-inverted- mesa (c) bi-mesa and (d) bi-inverted-mesa. (Adapted from [122])	28
Figure 23. convex-shaped structures: (a) plano-convex and (b) bi-convex. (Adapted from [122])	29
Figure 24. Crystals used in experimental tests	30
Figure 25. Holder developed (Static Cell)	31
Figure 26. Holder developed (Fluidic Cell in parts)	31
Figure 27. Holder developed (Fluidic Cell assembled)	32
Figure 28. Proposed design for acquisition circuit	33
Figure 29. Mesh of crystal geometry in COMSOL Multiphysics. The enlarged image corresponds to the mesh in thickness axis	34
Figure 30. Multiphysics simulation in COMSOL	34
Figure 31. Results for Multiphysics simulation – Conductance and Susceptance response	35
Figure 32. Equivalent electric circuit BVD used in Simulations	36
Figure 33. Results for BVD model simulation – Admittance response	36
Figure 34. Conductance for a crystal undamped. G_q curve was calculated without external influence. G_{total} was calculated considering G_{in} influence.	39
Figure 35. Susceptance for a crystal undamped. B_q curve was calculated without external influence. B_{total} was calculated considering the C_0 and B_{in} influence.	40

Figure 36. Conductance (G_{total}), Susceptance (B_{total}) and Admittance (Y_{total}) for a crystal undamped.	40
Figure 37. Conductance for a crystal in contact with water. $G_{q+sample}$ curve was calculated without external circuit influence. G_{total} was calculated considering G_{in} influence.....	41
Figure 38. Susceptance for a crystal in contact with water. $B_{q+sample}$ curve was calculated without the external circuit influence. B_{total} was calculated considering the C_0 and B_{in} influence.	41
Figure 39. Conductance (G_{total}), Susceptance (B_{total}) and Admittance (Y_{total}) for a crystal in contact with water.	42
Figure 40. User Interface for Matlab Simulations.....	43
Figure 41. Matlab simulation for water deposition on quartz crystal surface	43
Figure 42. General Scheme of the prototype first version.	44
Figure 43. Circuit schematic of the first version of the electronic acquisition system. (reproduced from [37]).....	45
Figure 44. Adjustment of the conductance and susceptance curves in Gaussian and sum of sine functions respectively. (reproduced from [37]).....	47
Figure 45. Curves for undamped crystal. Magnitude of Conductance $ G $ (green), Magnitude of Susceptance $ B $ (blue), Admittance Phase curve (red). (reproduced from [37]).....	48
Figure 46. Curves for crystal in contact with water. Magnitude of Conductance $ G $ (green), Magnitude of Susceptance $ B $ (blue), Admittance Phase curve (red), First Derivative of $ G $ curve (Turquoise). (reproduced from [37])	48
Figure 47. Electric field lines into the quartz crystal resonator.....	49
Figure 48. General Scheme of the prototype second version.....	50
Figure 49. Labview software developed for communication with arduino card.....	51
Figure 50. Labview software developed for processing data.....	53
Figure 51. User Interface to show the results.....	53
Figure 52. Adapter	54
Figure 53. Schematic for the adapter	54
Figure 54. General Scheme of the prototype final version.....	54
Figure 55. Scheme for communication with AD9850 DDS module and digital resistor	55
Figure 56. final version of the user interface.....	56
Figure 57. Variables to configure in final user interface.....	56
Figure 58. Panel to show results.....	57
Figure 59. Multichannel schematic in final prototype	57
Figure 60. Representation of the current signal acquired in the three possible cases according to the auto-gain adjustment system.	58
Figure 61. Crystal frequencies response in contact with water (First use).....	62
Figure 62. Crystal frequencies response in contact with water (months of use).....	62
Figure 63. Crystal Rhos (Γ) response in contact with water (First use).....	62
Figure 64. Crystal Rho's (Γ) response in contact with water (months of use).....	63
Figure 65. Δf_s and $\Delta \Gamma_{left}$ behavior for glycerol dilution samples.....	65
Figure 66. Response of the QCR prototype vs Fungilab reference.....	66
Figure 67. Biosensor response at different concentration sample.....	67
Figure 68. Model proposed including the parallel capacitance related to the fluid sample	68
Figure 69. Apparent viscosity obtained applying the Rouse model for white egg samples.....	73
Figure 70. Apparent Viscosity obtained by Rouse model vs viscometer measures.	75

TABLE INDEX

Table 1. Typical values of the quartz crystal equivalent circuit components (adapted from [81])	24
Table 2. Results for Multiphysics simulation in COMSOL	35
Table 3. Results for BVD Model using COMSOL.	37
Table 4. Results for BVD Model without external influences using Kirchhoff laws.	42
Table 5. Results for BVD Model considering external influences and using Kirchhoff laws. ...	42
Table 6. Results for BVD Model with external influences using Matlab.	43
Table 7. Glycerol dilutions data	60
Table 8. Experimental results for undamped crystal (first use).....	60
Table 9. Experimental results for undamped crystal (months of use).....	60
Table 10. Experimental results for crystal in contact with water (first use).....	61
Table 11. Experimental results for crystal in contact with water (months of use).....	61
Table 12. “Deltas” of the experimental results (first use)	61
Table 13. “Deltas” of the experimental results (months of use).....	61
Table 14. Comparison between experimental and simulation results for undamped crystal	63
Table 15. Comparison between experimental and simulation results for crystal in contact with water.....	64
Table 16. Comparison between experimental and simulation “deltas”	64
Table 17. Viscosity results for glycerol aqueous dilutions	65
Table 18. Comparison between QCR viscosity results and Fungilab viscometer.....	66
Table 19. Results for relative permittivity measures for water	68
Table 20. Macroscopic classification of synovial samples	71
Table 21. Data for white egg samples (average $\pm$ SD).....	72
Table 22. Constants used for obtaining correction factor χ	72
Table 23. Principal variables for obtaining the correction factor χ	72
Table 24. Results for egg white dilutions without and with Rouse Model factor correction.....	73
Table 25. Constants used for obtaining correction factor χ for HA dilutions.	74
Table 26. Hyaluronic Acid (HA) results and Zero-shear viscosity (Average $\pm$ SD).....	74
Table 27. Principal variables for obtaining the correction factor χ for HA dilutions.....	74
Table 28. Δf_s measured and adjusted with correction factor χ	75
Table 29. Results for HA dilutions without and with Rouse Model factor correction.....	75
Table 30. Additional results for HA dilutions using QCR prototype.....	76

1. INTRODUCTION

Quartz crystal resonator (QCR) is a surface acoustic wave transducer used as analytical instrument in complex media. There are wide applications for the QCR, the first one and more traditional is the gravimetric application called quartz crystal microbalance (QCM). The bases of the gravimetric applications were published by Günter Sauerbrey from 1959 [1]. Its use in vacuum is routine but this fact is not usually mentioned in the scientific literature [3].

In the 1980s Kanazawa et al, proposed a new non-gravimetric application to characterize liquids in contact with the surface of the crystal (active electrode) [2]. The possibility to use the QCR with liquids opened new investigation lines and made these transducers more attractive for biomedical applications because all biological samples need a liquid media to conserve its properties.

The widespread use of the QCM in liquids since 1990 was mainly driven by the novel instrumentation, but there also was an extensive interest in soft matter at interfaces. Currently, there are many biomedical applications using QCR's as transducers. For example: blood coagulation analysis [14–18], Alzheimer disease[19,20], Cancer[21–24] and pathogen detection [25–28]. Also, many environmental monitoring applications with QCR's. For instance: humidity sensors have special attractive because of their wide and essential applications in both industrial and domestic environments [29–32]. There are also studies in liquid quality [33–35], liquids characteristics [36–39] and gas detection [40,41].

When applying these quartz crystal sensors in gaseous media, a large set of circuits is available, which can be adapted to particular applications. But, in liquid applications, there is a viscous damping accompanied by a significant loss in the Q factor of the resonator requires specific solutions. Especially, the series resonance frequency and motional resistance are very important factors to determinate. In addition, there are many works where a parallel capacitance compensation is used. In particular, in this work the damping due to the liquid sample is compensated increasing the amplitude of the input sinusoidal signal. In this way the acquired signals recover sufficient amplitude to be interpreted.

The first chapter of this work contains the fundamentals of the QCR operation both in gases and liquid media. Furthermore, network analysis, impulse excitation technique, oscillators and lock-in techniques are commented. Impedance analysis especially allows a more complete characterization of the sensor and can nowadays be realized with sensor interface circuitry. The performance of electrical circuitry depends essentially on the stability of the acoustic device. However, in the proposed design, the Lock-in fundament is used, especially for obtaining the susceptance of the crystal according to Nakamoto proposal[42]. In this first chapter is also presented concepts related to viscoelastic behavior of the samples used. Especially, the model Rouse was used to model the behavior of the pseudoplastic samples.

The second chapter contains the detailed information about the design and construction of the prototype of the biosensor. In this chapter is presented the simulation results based on geometric simulations using COMSOL Multiphysics and the BVD model commonly used to explain resonator systems. The simulations results were compared with initials experimental results and the corresponding analysis is done. It is important to say, although the QCR is the transductor element, “the heart of the piezoelectric technique” we have to consider the necessity and the effect

of some electronic instruments for obtaining the electrical signals and register the interactions between the electrode surface and the samples. In particular, the simulations just consider the crystal behavior but in real world there are other electronic components that affect the crystal response. In this work, the external influences of these additional electronic components are added to the BVD model in order to close the simulations results with experimental results.

The third chapter contains the biomedical application proposed and the experimental results based on the measures of pseudoplastic samples (egg white and hyaluronic acid dilutions). The biomedical application is related to the use of QCR prototype designed as an electronic instrument to characterize synovial fluid samples in order to detect arthritic diseases. Due to difficulty in obtain real samples of healthy and abnormal samples of synovial fluid, dilutions of hyaluronic acid at different concentrations related to specific disease were elaborated. These results are shown in this chapter.

Finally, the main conclusions about the work done are presented including future perspectives related to the use of QCR as viscosity sensor.

2. PRESENT PROBLEMATIC AND HYPOTHESIS

According to the World Health Organization[43] today the people live longer, but it is not necessarily healthier than before: almost a quarter (23%) of the overall global burden of mortality and morbidity is recorded in over 60 years, and much of that burden can be attributed to long-term illnesses such as cancer, chronic respiratory diseases, heart disease, musculoskeletal diseases (such as arthritis and osteoporosis) and mental and neurological disorders.

The joint pain is the second leading cause of disability after respiratory problems and it is the leading cause of chronic disease in the general population. More than 45 million adults have been diagnosed with arthritic disease in North America. Adults of working age (18-64 years) have a condition related to arthritis, 31% of labor constraints are related to arthritic or rheumatic symptoms. Due to the arthritic disease affects the musculoskeletal condition it has a significant component in labor productivity and quality of life of sufferers. About 25% of the treatments carried out in social security in America are related to a rheumatic condition [7].

According to the Spanish Society of Rheumatology (SER), in Spain more than 200.000 people suffer from rheumatoid arthritis and each year, around 20.000 new cases are diagnosed. A critical view if we consider that rheumatoid arthritis is the most disabling rheumatic disease [44].

In Latin America, the scenario includes chronic degenerative diseases and especially accompanied by scarce resources used for meeting these needs. In consequence, the health care is postponed and the rheumatic diseases are more prevalent. Both in Spain and Latin America, the prevalence of patients with musculoskeletal pain who have appealed to medical care are often attended by a general doctor, who in most of cases, prescribe NSAIDs (Nonsteroidal anti-inflammatory drugs). Therefore, several diseases that should be managed by the rheumatologist there are not diagnosed in time [45].

Considering this scenario, in this thesis, the design of a biosensor for contributing to the diagnosis of arthritic diseases is proposed. The biosensor based on quartz crystal resonator (QCR) is used to characterize samples whose behavior is similar to the synovial fluid. Synovial fluid is a mixture of plasma and hyaluronic acid dialysate with pseudoplastic behavior. The main function of the synovial fluid is being a lubricant in the joints keeping a minimum friction between the bones, especially during movement [4–7]. Because of this lubricating function, the synovial fluid analysis is important for the detection and diagnosis of arthritic diseases. In particular, the decrease of the hyaluronic acid in the synovial fluid has been associated with arthritic disease because the lubricating properties of synovial fluid and its viscosity depend on the concentration of the hyaluronic acid [4–8]. Moreover, the relationship between changes in the viscosity of the synovial fluid and the joint affections is widely documented [4–13].

Based on the above, the main goal of the QCR biosensor is to provide additional tools to the diagnosis of arthritic diseases such as osteoarthritis or rheumatoid arthritis through the analysis of synovial fluid viscosity.

3. GOALS

- To design a low-cost biosensor based on quartz crystal resonator capable of characterizing biological samples according to their viscosities in a simple and reliable way.
- To design a software with intuitive user interface capable to be used in any computer to control the sensor from any place (point of care sensing)
- To evaluate the biosensor proposed with reference samples (Newtonian fluids) to obtain its sensibility, resolution and to confirm the reproducibility of the measurements.
- To evaluate the biosensor proposed with pseudoplastic samples (Non-Newtonian fluids) to know the performance of the sensor with similar samples to synovial fluid.
- To propose additional tools in the diagnosis of arthritic diseases with the aim of detecting these abnormal conditions on time and in an effective way.

4.CHAPTER 1 - Fundaments and QCR Theory

The variables of interest in the QCR related to the interactions between the crystal surface and the sample are the series resonance frequency (F_s), the half band half width (Γ), the frequency at minimum impedance with zero phase (F_r), and the series motional resistance (R_s). These variables are shown in Figure 1.

The series resonance frequency F_s can be defined as the frequency where the conductance has a maximum value. The frequency at minimum impedance F_r where the susceptance value is zero. The motional resistance R_s is defined as the inverse value of the conductance at series resonance frequency. R_s corresponds with the series equivalent resistance in the electrical model (BVD).

According to the circuits theory, the value F_s and F_r in a resonant circuit are the same because at resonance frequency its imaginary component is zero and its real component achieves the maximum value. Although, in the practice, F_s and F_r are not the same value because there is a capacitive effect between the electrodes of the crystal (C_0) and the external circuits connected to the crystal cause additional capacitances. In addition, the section 6.11 shows how the sample deposited on the crystal adds other capacitive component to the measure. This component causes an additional shift between F_s and F_r .

Figure 1. Frequency response of the crystal admittance

The values of ΔF and $\Delta \Gamma$ represent the changes in the behavior of the crystal due to the properties of the sample and these are obtained using the equation 1, 2 and 3.

$$\Delta F = F_s' - F_s \quad (1)$$

$$\Delta \Gamma = \Gamma' - \Gamma \quad (2)$$

$$\Delta R_s = R_s' / R_s \quad (3)$$

If a thin film of sample is deposited on an electrode of the crystal and this sample has similar acoustic properties of the quartz, this deposition can be seen like an increase of the acoustic thickness of the crystal (Figure 2).

Figure 2. Increase of the crystal thickness due to the mass deposition. (reproduced from [3])

According to Figure 2, the wavelength of sound (λ) increases and the resonance frequency (F_s) decreases. The phenomenon is explained with the following equation:

$$F_s = \frac{v_q}{\lambda}; \lambda = 2(d_q + d_f) \quad (4)$$

Where;

v_q is the speed of sound and d_q and d_f are the thickness of the quartz plate and the sample film respectively.

In other words, to deposit a thin film of sample with similar acoustic properties of the quartz is equivalent to “increase” the thickness of the quartz crystal. This effect is the operating principle of the Quartz Crystal Microbalance (QCM). The Sauerbrey equation (equation 5) shows the same behavior:

$$\Delta F = -\frac{2f_0^2}{A\sqrt{\rho_q\mu_q}}\Delta m \quad (5)$$

$$F_0 = \frac{n}{2d_q} \left(\sqrt{\mu_q/\rho_q} \right) \quad (6)$$

Where ρ_q and μ_q are the specific density and the shear modulus in quartz, respectively, f_0 is the fundamental series resonance frequency in the quartz, related to its thickness d_q , Δm is the thin film mass deposited, A is the piezoelectrically active crystal area and n is the overtone number.

It is important to note that Sauerbrey equation does not consider the half band half width (Γ). The reason is the Sauerbrey equation only applies when $|\Delta\Gamma| \ll |\Delta F|$. This relation occurs when the sample film is rigid and its environment is not liquid. In consequence, when the crystal surface is in contact with liquids and the application is gravimetric, for instance: cell culture or protein detection; the frequency shift due to mass deposition and the frequency shift due to contact with the liquid are accumulative [3].

In these cases, the measure of the frequency shift is not sufficient to fully characterize the acoustic response of a quartz crystal with sample. Any other variable must be considered to quantify the energy dissipation. The half-band-half-width Γ is convenient for this purpose [46].

In addition, the viscoelastic properties of macromolecular coating materials have a strong impact on its vibration and further diminish quality factor (Q). When the QCR sensor is in contact with

liquid media, the oscillation is significantly damped too and again the quality factor is significantly decreased.

With the aim of explaining QCR behavior, many theoretical models have been developed, especially at their resonance frequency and near frequency ranges. In particular, the equivalent electrical model BVD has been tested as a useful tool.

4.1. Equivalent electrical model BVD

When an AC voltage is applied between the electrodes of the QCR, a shear force is induced generating a transversal acoustic wave propagating through the thickness of the quartz. This wave penetrates the medium in contact with it. The mechanical interaction between the resonator and the sample film modifies the electrical response (impedance) of the crystal. This phenomenon enables the resonator as a sensor to detect changes in the properties of sample in contact with it.

To analyze the quartz as an electronic component through a circuit is useful to represent the sensor model by suitable impedances.

The equivalent circuit Butterworth-Van-Dyke (BVD) is widely used in the literature to represents the behavior of the quartz crystal close to resonance frequency (Figure 3)[37,47–50].

Figure 3. Butterworth-Van-Dyke equivalent circuit for undamped crystal. (adapted from [37])

In the Figure 3, the up-hand branch (motional arm) describes the mechanical behavior of the quartz crystal resonator. The electrical model consists in a motional resistance R_q , an equivalent inductance L_q and an equivalent capacitance C_q . R_q describes the losses in the crystal. L_q is related to the displaced mass. C_q is analogous to an elastic modulus. The parasitic capacitance, C_0 , on the down-hand branch results from the dielectric properties of the quartz disk and interconnection capacitances of the external circuit.[50,51]

The resonance frequency in BVD circuit is calculated as:

$$F_s = \frac{1}{2\pi\sqrt{L_q C_q}}; \quad (7)$$

The half band half width (Γ_s) is calculated as:

$$\Gamma = \frac{R_q}{4\pi L_q}; \quad (8)$$

Q factor is calculated as:

$$Q = \frac{F_s}{2\Gamma}; \quad (9)$$

According to Figure 3, the equivalent admittance at BVD circuit can be written as:

$$Y_q = j\omega C_0 + \left(\frac{1}{R_q + j\omega L_q + 1/j\omega C_q} \right); \quad (10)$$

Where the motional admittance Y_m is defined as:

$$Y_m = \left(\frac{1}{R_q + j\omega L_q + 1/j\omega C_q} \right); \quad (11)$$

Due to the sample in contact with the crystal electrode generates a mechanic load effect (Z_L), the electric components used to model the sample effect are in series to the motional arm of the model (Figure 4).

Figure 4. Butterworth-Van-Dyke equivalent circuit for damped crystal. (adapted from [37])

When the sample layer is rigid mass, the electric component to model the mechanic load effect is an inductance: $Z_L = L_{\text{mass}}$

When the sample layer is liquid, the electric component to model the mechanic load effect are a series of inductance and resistance $Z_L = L_{\text{liquid}} + R_{\text{liquid}}$

For obtaining the values of the components of the model, it is necessary a mathematical analysis for relating the physical properties of the quartz and the sample with the BVD model. This analysis can be shown in [52]. To do the analysis, Granstaff and coworkers [52] have used the Mason model as described in Rosenbaum [53,54].

Mason model uses the theory of transmission lines to represent the physical behavior of the crystal and the samples layers in one dimension. The model distinguishes between piezoelectric and non-piezoelectric materials. The non-piezoelectric film is mathematically represented by a 2x2 matrix and physical represented by a two-port circuit [52,53]. The piezoelectric material is also represented by transmission lines. Granstaff et al, used a circuit with two acoustic ports and one electrical port [52,53].

In Mason model the acoustical variables are analogous to electrical ones: the force corresponds to voltage, particle velocity corresponds to current and mechanical impedance (Force/particle velocity) corresponds to electrical impedance (Voltage/current).

For piezoelectric and non-piezoelectric models, the mathematic implementation according to Rosenbaum [53] and Granstaff [52], is a matrix equation to express the outputs (current and voltage) as a linear function of the input (current and voltage) and the acoustic parameters of the film sample.

$$\begin{bmatrix} V_i \\ I_i \end{bmatrix} = \begin{bmatrix} A & B \\ C & D \end{bmatrix} \begin{bmatrix} V_o \\ I_o \end{bmatrix} \quad (12)$$

For a non-piezoelectric layer:

$$A = \cos(\hat{k}d); \quad (13)$$

$$B = j * Z_q \sin(\hat{k}d); \quad (14)$$

$$C = j * \sin(\hat{k}d)/Z_q; \quad (15)$$

$$D = \cos(\hat{k}d); \quad (16)$$

$$\hat{k} = \omega\sqrt{\rho/G} \quad (17)$$

$$Z = \sqrt{\rho G} \quad (18)$$

For a piezoelectric layer:

$$A_q = \left[\frac{j\phi^2}{\omega C_0} + A(a+b) \right] / \phi a; \quad (19)$$

$$B_q = \left[\frac{2j\phi^2 a}{\omega C_0} + A(a^2 + 2ab) \right] / \phi a; \quad (20)$$

$$C_q = j\omega C_0 A(a+b) / \phi a; \quad (21)$$

$$D_q = j\omega C_0 A(a^2 + 2ab) / \phi a; \quad (22)$$

$$a = jZ \tan(\hat{k}_q d_q / 2); \quad (23)$$

$$b = -jZ / \sin(\hat{k}_q d_q); \quad (24)$$

$$\hat{k}_q = \omega\sqrt{\rho_q/G_q} \quad (25)$$

$$Z_q = \sqrt{\rho_q G_q} \quad (26)$$

$$C_0 = \epsilon_{22} A / d_q; \quad (27)$$

$$\phi = (e_{26} / \epsilon_{22}) C_0; \quad (28)$$

$\hat{k}$ is the complex propagation constant and d is the thickness of the layer. Z is the acoustic impedance of the layer. C0 is the static capacitance of the crystal; ϵ_{22} is the quartz permittivity; A is the active area of the electrode; G_q is the complex shear modulus of the quartz; ϕ is the ratio of the conversion between the electrical and the acoustical variables; e_{26} is the piezoelectric stress constant. The index (q) is related to quartz crystal.

The admittance for the quartz crystal can be written as:

$$Y_q = \frac{C_q V_q + D_q I_q}{A_q V_q + B_q I_q} = \frac{C_q Z_s + D_q}{A_q Z_s + B_q}; \quad (29)$$

Where: Z_s is the impedance seen at the crystal in its surface. In other words, is the impedance at the quartz-layer interface.[52]

For a bare crystal $Z_s = 0$ because the top surface is stress free. For a crystal in contact with a single layer, the equation 12 is solved for a non-piezoelectric layer obtaining:

$$Z_s = \sqrt{\rho G} \tanh(j\hat{k}d); \quad (30)$$

When the single layer is a thin mass layer: $\tanh(j\hat{k}d) \rightarrow j\hat{k}d$. [52] Solving equation 30 using equation 17:

$$Z_s = j\omega \rho d_f = j\omega \rho_s; \quad (31)$$

Where d_f is the mass film thickness and ρ_s is the material's density per area. The expression ρd is written as ρ_s because the thickness d_f is an unknown value.

When the single layer is a Newtonian fluid: $G = j\omega\eta$ and $\tanh(j\hat{k}d) \rightarrow 1$. [52] Solving equation 30 using equation 17:

$$Z_s = \sqrt{j\omega\rho\eta}; \quad (32)$$

Comparing the admittance obtained with the admittance of the electrical model (BVD):

$$Y_q = \frac{C_q Z_s + D_q}{A_q Z_s + B_q} = j\omega C_0 \left(1 + \frac{Y_m}{j\omega C_0}\right); \quad (33)$$

Where: $Z_m = 1/Y_m$. Solving to Z_m :

$$Z_m = \frac{A_q Z_s + B_q}{D_q - j\omega C_0 B_q + Z_s (C_q - j\omega C_0 A_q)}; \quad (34)$$

Replacing Z_s for a bare crystal in the equation 34 and using the equations 19 to 28 can be obtained the equations by inductance (L), resistance (R), capacitance (C) components in BVD model (figure 3).

$$C = \frac{8e_{26}^2 A}{(N\pi)^2 G_q d_q} \quad (35)$$

$$L = \frac{1}{\omega^2 C} \quad (36)$$

$$R = \frac{\eta_q}{G_q C} \quad (37)$$

Replacing Z_s for a crystal in contact with a mass layer (equation 31 into equation 34), the components of the model BVD are the same L-R-C for the quartz crystal plus one inductance L_{mass} to model the mass effect in the crystal:

$$L_{mass} = \frac{2\omega * L \rho_s}{N\pi} * \sqrt{\left(\frac{1}{\rho_q G_q}\right)} \quad (38)$$

In case of the crystal in contact with liquid layer (equation 32 into equation 34) the components are the same L-R-C for the quartz crystal plus one inductance L_{liq} and R_{liq} to model the liquid effect in the crystal:

$$L_{liq} = \frac{\omega * L}{N\pi} * \sqrt{\left(\frac{2\rho_l \eta_l}{\omega \rho_q G_q}\right)} \quad (39)$$

$$R_{liq} = \frac{\omega * L}{N\pi} * \sqrt{\left(\frac{2\omega \rho_l \eta_l}{\rho_q G_q}\right)} \quad (40)$$

In addition, for liquid applications (quartz crystal with single side contact with a liquid) the quality factor Q is given by:

$$Q = \frac{\omega(L_q + L_{liq})}{R_q + R_{liq}} \quad (41)$$

The additive contributions to L_q and R_q are similar because the real and imaginary parts of Z_L are similar too. As $L_q \gg L_{liq}$ and $R_q \ll R_{liq}$, the quality factor Q changes inversely with $\sqrt{F_0}$ [55]. This phenomenon can be observed in the plot of the conductance (G) - Figure 1.

Using the typical nomenclature, $(R_q + R_{sample})$ is called R_s and $(L_q + L_{sample})$ is called L_s . R_s can be obtained with the inverse of the maximum value of the conductance curve. For undamped crystal (without sample) $R_q = R_s$ and $L_q = L_s$. For the following sections, the load due to the sample (mass or liquid) will be denote with sub-index "s"

The equivalent circuit BVD also permits to identify the real and imaginary components of the crystal admittance. Using these components is possible to obtain the locus diagram of admittance $Y = G + jB$.

Figure 5. Locus diagram of the crystal admittance. (Adapted from [56])

According to Figure 5, there are three representative resonant frequencies (F_m , F_s and F_r). The first one (F_m) is obtained when the admittance value (Y) of the resonator is maximum. The second one (F_s) is obtained when the conductance value (G) of the resonator is maximum. This frequency is denominated “series resonant frequency”. The last one (F_r) is obtained when the susceptance value (B) is zero. This frequency is called “frequency at minimum impedance with zero phase” [37,55,57].

For a bare crystal (without sample), the real part of the crystal impedance R_s is very small; in consequence, the difference between the representative frequencies is not evident. However, when the sample is deposited, the damping generated on the crystal is related to the increase of the R_s and the difference between the frequencies is more obvious [3,47,55].

According to circuit theory, the resonant frequency for an ideal resonator is:

$$F_s = \frac{1}{2\pi\sqrt{L_s C_s}} \quad (42)$$

According to Wolfbeis [55], for small R_s , the characteristic frequency (F_r) may be calculated as

$$F_r \approx \frac{1}{2\pi\sqrt{L_s C_s}} + \frac{R_s^2 C_0}{L_s^2 C_s} \quad (43)$$

It is important to note that F_r depends on the equivalent resistance R_s .

The only frequency independent of the influence of the capacitance C_0 is F_s . F_m and F_r depend on the C_0 value. In addition, external capacitances due to the external circuits connected to the crystal amplify the deviation between F_m and F_r with F_s [47,55].

4.2. QCR in contact with fluids

In accordance with previous sections, when the crystal surface is in contact with liquid sample, there is a high damping in the crystal vibration. This damping can be observed in the decay characteristic length (δ).

$$\delta = \sqrt{\frac{2\eta_L}{\omega\rho_L}} \quad (44)$$

Where ρ_L and η_L are the density and viscosity in fluid respectively. Equation 44 shows the decay characteristic length as proportional to the ratio viscosity to density of the liquid and as inversely proportional to the angular frequency (ω) [58]. For 10MHz AT cut quartz crystal this length is: $\delta=178\text{nm}$. That is mean, the acoustic wave generating for the crystal propagated into the fluid is completely attenuated at 178nm measured from crystal surface. In consequence, quartz crystal resonators respond to the fluid that is present in regions very near to its surface [15].

Therefore, the vibration of that crystal depends on the properties of that fluid. In particular, the resonance frequency of the crystal is related to the viscosity and the density of the fluid. The density is usually known with external equipment, for example: analytical balance; therefore, the response of the crystal contributes to obtain the value of the sample's viscosity.

The viscosity is the property of fluids which indicates resistance to flow. In biomedical applications, the viscosity of the fluid is a relevant parameter, which determines phenomena related to some diseases. For example, the blood viscosity is a relevant variable in several diseases such as ischemic heart disease, arterial or venous occlusion and hypertension. Moreover, the decrease of the viscosity in the synovial fluid is widely related to arthritic disease. Therefore, accurately measuring viscosity of a certain fluid may be a method for diagnosing a certain disease.

Figure 6. Basic concepts for viscosity theory (reproduced from [59])

According to Figure 6, the viscosity (η) is defined as:

$$\eta = \frac{\text{Shear stress}}{\text{Strain rate}} \quad (45)$$

Where:

Shear stress is the applied force divided by the area parallel to the force (F/A). strain rate is the velocity per unit height (V/H)

The resonance frequency can be defined as a complex value (equation 46) using the series resonance frequency and the half-band-half-width described in the CHAPTER 1 - Fundamentals and QCR Theory:

$$\tilde{F} = f_s + i\Gamma \quad (46)$$

According to Johannsmann, the relationship between Δf and the viscosity of a deposited fluid over a quartz crystal can be written as [3]:

$$\frac{\Delta f + i\Delta\Gamma}{f_0} = \frac{-1+i}{\sqrt{2}\pi Z_q} * \sqrt{\omega} \sqrt{\rho_L(\eta'_L - i\eta''_L)} \quad (47)$$

Where:

Z_q : is the acoustic impedance of the quartz crystal. $Z_q = \sqrt{\rho_q * G_q}$

ω : is the angular frequency $\omega = 2\pi f$

ρ_L and η_L are the density and viscosity of the fluid respectively.

For Newtonian fluids ($\eta'_L = \text{const}$, $\eta''_L = 0$). For Fluids with viscoelastic behavior ($\eta'_L = \eta(\omega)$, $\eta''_L \neq 0$).

$$\eta'_L = \frac{G'_L}{\omega} = -\frac{\pi\rho_q G_q}{\rho_L f_r} \frac{\Delta F \Delta \Gamma}{f_0^2} \quad (48)$$

$$\eta''_L = \frac{G''_L}{\omega} = \frac{1}{2} \frac{\pi\rho_q G_q}{\rho_L f_r} \frac{(\Delta \Gamma^2 - \Delta F^2)}{f_0^2} \quad (49)$$

4.3. Newtonian Fluids

In Newtonian fluids, the viscous stress arising from its flow, at every point, is constant. In other words, the viscosity is independent of shear strain rate. Solving the equation 47 for viscosity considering $\eta'_L = \text{const}$, $\eta''_L = 0$, the equation 49 is obtained:

$$\eta'_L = \frac{\pi Z_q^2 \Delta f^2}{f_0^2 \rho_L f} = \frac{\pi Z_q^2 \Delta \Gamma^2}{f_0^2 \rho_L f} \quad (50)$$

Equation 50 suggests that $\Delta f = \Delta \Gamma$ for Newtonian fluids. Therefore, for Newtonian fluids, it is not necessary to obtain both values, Δf is sufficient to know the viscosity sample.

At resonance frequency $f=f_0$, then:

$$\eta'_L = \frac{\pi Z_q^2 \Delta f^2}{f_0^3 \rho_L} \quad (51)$$

$$\Delta f = -\sqrt{n} f_0^{3/2} \sqrt{\frac{\rho_L \eta_L}{\pi \rho_q G_q}} \quad (52)$$

The equation 51 shows the results of Gordon-Kanazawa equivalent to equation 52 (Kanazawa equation):

In consequence, for Newtonian fluids, the relation between Δf and η_L is explained by Kanazawa equation. It is important taking into account, the Newtonian behavior is related with low molecular weight [60].

4.4. Non-Newtonian Fluids

A non-Newtonian fluid is a fluid whose properties differ in any way from Newtonian fluids. In particular, the viscosity of non-Newtonian fluids is dependent on shear rate.

Figure 7. Typical flow curves for Newtonian, shear thinning and shear thickening (dilatant) fluids: (a) shear stress as a function of shear rate; (b) viscosity as a function of shear rate (reproduced from [61]).

There are two typical behavior for non-Newtonian fluids (shear thickening and shear thinning) [59,62]. Figure 7 shows the behavior for these fluids according to the shear strain rate.

A shear thickening fluid or “dilatant” is a Non-Newtonian fluid whose viscosity increases with the increase of the applied shear stress [63]. This behavior is controlled by several factors as: particle size, shape, and distribution. Shear thickening behavior occurs when a colloidal suspension transitions from a stable state to a state of “flocculation”. In other words, the fluid is colloiddally unstable due to the formation of aggregates larger than colloidal size. They are rarely encountered, but, one example is an uncooked paste of cornstarch and water [62].

Shear-thinning fluids (Pseudoplastic fluids) have a lower apparent viscosity at higher shear rates, and are usually solutions of large polymeric molecules in a solvent with smaller molecules or colloids. It is supposed that the large molecular chains tumble at random and affect large volumes of fluid under low shear, but they gradually align themselves in the direction of the increase of the shear rate producing less resistance [59,62,64].

Figure 8 shows the cross-sectional area of two different molecules. At low flow rates, molecules with conformations long and thin (Figure 8 – Blue) have large cross area due to them tumbling in solution but at high shear rate the molecules align with the flow, giving much smaller effective cross area and hence much lower viscosities (Figure 9 - red). More compact molecules (Figure 8 – red) are not so much affected by their orientation relative to flow and their viscosity do not change so much with the increase of the shear rate (Figure 9 - blue) [59,62].

Figure 8. Different molecule conformations in polymer dilutions

Figure 9. Viscosity behavior according the molecule conformation. (adapted from [59])

According to Figure 9, hydrocolloid solutions exhibit non-Newtonian behavior depending on the concentration of the polymer in solution and their viscosity depends on the shear strain rate, typically as opposite. where $\dot{\gamma}$ is the shear strain rate, η_0 and η_∞ are the viscosities at zero and infinite shear strain rate respectively and τ is a shear-dependent time constant that represents the reciprocal of the shear strain rate required to halve the viscosity [60].

In addition, at low oscillation frequencies (low shear strain rate), the polymer chains can release stress via deformation and disentanglement; hence, the polymer such as synovial fluid behaves more like a viscous fluid (with the viscous modulus G'' greater than the elastic modulus G'). At higher oscillation frequencies (high shear strain rate), the polymer exhibits stronger elastic behavior (with $G' > G''$), because the polymer chains are not sufficiently agile to deform and disentangle in response to rapid change in stress [65].

With the aim of analyzing the pseudoplastic fluids with QCR, it is important considering that the shear rate for quartz crystal resonators are many times higher than any conventional rheometer. Therefore, when a pseudoplastic sample is deposited and characterized using a QCR, the frequency shift obtained is many times lower than expected. [66,67] In consequence, the equation 46 cannot be applied because the apparent viscosity obtained will be similar to the viscosity of the sample's solvent [29].

The samples analyzed in this work are classified as hydrocolloids with shear thinning behavior and viscoelastic properties (Chapter III – The Proposed Biomedical Application). The samples consist in hyaluronic acid dilutions at different concentrations in order to emulate the behavior of the synovial fluid as a polymeric mixture [7,8,68,69].

However, for an adequate analysis of the QCR response, it is necessary to consider a model to explain the polymer behavior into the solvent. For this purpose, the Rouse model has been chosen due to its simplicity explaining the polymer chains interactions and the changes in the chains conformation with time.

4.5. Rouse Model

The model assumes the polymer beads have no excluded volume (they are essentially a point) and that there are no hydrodynamic interactions between the beads. These assumptions generate some inaccuracies in the model.[70] In this model, the elastic forces on the any bead are exerted by the two springs that connect the adjacent beads, as shown in Figure 10. One spring between the beads pulls each bead with a certain force. Likewise, the other spring pulls the bead with the same force

magnitude with another direction. In addition, all beads receive a random force that changes with time from nearby solvent molecules.[70]

Figure 10. Polymer chain conformation according to Rouse Model (reproduced from [70])

Figure 10 shows a polymer chain of a bead-spring model changes its conformation with time. According with [66], Rouse model provides a good representation of the linear viscoelastic response of pure linear polymers.

In the Rouse model, the relaxation time is defined as[64,66,67]:

$$\tau_0 = \frac{6\eta_0 M}{\pi^2 c R T} \quad (53)$$

where M is the molecular weight of the polymer, R is the gas constant ($8.314 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$), T is the absolute temperature (288 K for experiments), η_0 is the zero-shear rate viscosity and c is the polymer concentration. According to [71] the zero shear rate viscosity η_0 is the viscosity of the liquid obtained in the limit of shear rate tending to zero. According to [72], the relaxation time is a characteristic time required for the polymer coil to relax from a deformed state to its equilibrium configuration. It is a key parameter for characterizing a viscoelastic fluid.

4.5.1. Constitutive Equations

There are several constitutive models for linear viscoelastic materials. These models include the Maxwell model, the Kelvin-Voigt model and the standard linear viscoelastic solid model that combines the two previous models. All these models decompose the tension and deformation in two summands, one that represents the elastic effects and another that represents the viscous effects, being these models, interpretable in terms of springs and dampers.

According to Maxwell constitutive equations (Appendix A) [66]:

$$\frac{\eta'}{\eta_0} = \frac{1}{\zeta(v)} \sum_{k=1}^{\infty} \frac{k^v}{k^{2v} + (\tau_0 \omega)^2} \quad (54)$$

$$\frac{\eta''}{\eta_0} = \frac{\tau_0 \omega}{\zeta(v)} \sum_{k=1}^{\infty} \frac{1}{k^{2v} + (\tau_0 \omega)^2} \quad (55)$$

In the Rouse model, $v=2$, $\zeta(v)$ is the Riemann Zeta Function and τ_0 is the relaxation time (equation 52).

$$\tan(\delta) = \frac{\eta'[\omega]}{\eta''[\omega]} = \frac{G''[\omega]}{G'[\omega]} \quad (56)$$

Where $\eta' = G''/\omega$ and $\eta'' = G'/\omega$. [3] Tan(δ) is the loss tangent. Loss tangent quantifies the balance between energy loss and storage. A value for tan(δ) greater than unity indicates more "liquid" properties, whereas one lower than unity means more "solid" properties [59].

According to Nwankwo and coworkers [66]:

The frequency of the resonator in contact with a linear viscoelastic fluid can be expressed as:

$$\omega = \omega_0 + \Delta\omega \quad (57)$$

Where ω_0 is the resonance frequency of the crystal without sample and $\Delta\omega$ is the frequency shift generated by the linear viscoelastic sample deposition. $\Delta\omega$ is a value experimentally obtained.

$$\omega_0 = \frac{n\pi}{h} \sqrt{\frac{\mu_q}{\rho_q}} \quad (58)$$

Where n is the overtone number. $\Delta\omega$ can be written as:

$$\Delta\omega = \Delta\omega_0 * \chi \quad (59)$$

Where χ is a correction for the effect of a finite relaxation time in the fluid on the observed frequency shift [66].

$$\chi = \sqrt{\frac{\eta'}{\eta_0}} \sqrt{\left(\frac{\sqrt{(1+\tan^2(\delta))-1}}{\tan(\delta)} \right)} \quad (60)$$

$\Delta\omega_0$ corresponds to Kanazawa and Gordon's obtained from a consideration of QCR damped by a Newtonian fluid. [66]

4.6. Hyaluronic Acid

Hyaluronic acid (HA) is a linear polysaccharide discovered by Karl Meyer and John Palmer in 1934. It consists of alternating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine linked by β -(1-3) and β -(1-4) bonds (Figure 11) [73]. Under physiological conditions, it behaves as a salt, hence the term hyaluronate. The molecule is commonly referred as hyaluronan.

Figure 11. Chemical Structure of HA (reproduced from [74])

HA is widely observed in eukaryotes. High concentrations are found in soft connective tissues such as skin, synovial fluid, umbilical cord and the vitreous body. Lung, kidney, brain and muscle also contain considerable amounts of HA[75].

In markets, there are available HA is extracted from animal source (rooster comb), or from bacteria, mostly (Streptococci). Because its chemical structure is source independent, exogenously applied HA is biocompatible and biodegradable. However, although the chemical

structure of HA is the same in all tissues, HA characteristics and impurities are dependent on the source, isolation and purification method [76]. In particular, in Colombia there are three different Hyaluronic acid compounds named: Synvisc, dropial and suplasin. The commercial name of this hyaluronic acid compounds is “viscosupplements” and they are commonly used in advance arthritic treatments as a synovial fluid substitutive. Viscosupplementation technique is the use of HA solutions to replace or supplement tissue fluids, usually pathological synovial fluid. Pathological synovial fluid differs from healthy synovial fluid because HA is degraded by radicals produced during inflammatory reactions[77]. The price of these viscosupplements varies between 250 and 300 eur per 6ml. The principal factor of difference in hyaluronic acid compounds is the molecular weight (Mw), commercially there are two groups of compounds; the first one with Mw around 1.5MDa and the second one with Mw around 8MDa. Due to accessibility and price, in this work the hyaluronic acid used has been bought with Mw=1.5MDa.

Between the functions of HA into the body, HA networks are highly resistant to water flow as the dynamics of the transient network are relatively slow. In consequence, HA is an efficient barrier against quick changes in water content[76,78]. Also, the HA network acts as a filter for other molecules. Small molecules can diffuse freely in the HA network, while larger molecules are slowed down or immobilized. This way, HA can regulate the transport of molecules through the interstitial space [76]. However, the most important function of HA is related to its viscoelasticity. Any solution of HA are viscoelastic due to chain entanglement of the polymer.[76] In addition, the viscosity depends on the shear rate (non-Newtonian and pseudoplastic behavior). The pseudoplastic behavior results useful because permits to HA has a low viscosity during rapid movements and a high viscosity during slow movements. This behavior is clear during the experimental section in chapter III[75].

In relation to the physical structure, HA performs as an extended, stiffened random coil in solution. This is the result of helix formation by stabilizing hydrogen bonds parallel to the axis of the chain. The helix also displays relatively hydrophobic regions formed by the axial hydrogen atoms. This could form the basis for chain-chain associations. The random coil conformation of HA in solution can be regarded as spherical with a radius of gyration of about 200nm (Figure 12)[76].

Figure 12. Physical conformation of HA, radius of gyration. (reproduced from [76])

HA solutions are viscoelastic and exhibit non-Newtonian behavior, specifically, pseudoplastic behavior. In accordance with section 4.4, pseudoplastic solutions means that the viscosity decreases with increasing shear rate [76]. This behavior is consequence of alignment of the chains, which makes the chains flow easier, this phenomenon is explained in section 4.4. At low strain frequencies, the viscous behavior dominates because the chains have enough time to take up their original conformation while the stress is being applied. At high strain frequencies, the behavior of the fluid is mainly elastic, because the frequency at which the strain is applied is faster than the time scale of topological interactions [76]. At low shear rates, the viscosity is shear-independent. There is a theory value called “zero-shear viscosity” which is defined as the viscosity at the limit of low shear rate. Zero-shear viscosity is also a qualitative indicator of molecular weight (M_w) of a dissolved polymer as in the case of this work. This value is taken by extrapolation of the viscosity plateau value which is obtained with a highly sensitive instrument that can generate very small stresses and measure very low shear rates. In particular, for this work, the viscosity plateau value has been obtained using the reference viscometer Fungilab Smart (Figure 13) and the spindle L2 for samples with high concentration and the spindle LCP for samples with low concentration (more diluted).

Figure 14 shows a viscosity profile obtained for very low values of shear rate. In accordance with this figure, the less value of shear rate of the Fungilab viscometer (0.3 RPM = 5mHz) is a sufficient low shear rate to obtain the viscosity plateau value. Now, considering at low shear rates, the viscosity is shear-independent. The viscosity plateau value has been used as the Zero Shear viscosity for effects of the results in Chapter III – The Proposed Biomedical Application: Potential diagnosis of arthritic disease.

Figure 13. Fungilab Smart Viscometer

Figure 14. Profile for the Zero Shear Viscosity of a HA dilution with low viscosity

4.7. Colloidal Dispersions

In accordance with previous sections, HA dilutions can be considered as colloidal dispersions. Colloidal dispersions can be defined as small particles embedded in a liquid [3]. According to Johannasman, one of the characteristic time scales in colloid science is the time needed for a rearrangement of the local cages around a particle [3]. This time is defined as:

$$T_{rearrangement} = \frac{d_p^2}{D} \quad (61)$$

Where d_p is the interparticle distance (often approximated by the particle diameter) and D is the particle diffusivity. If the measurement of the viscosity is done on a time scale faster than this time, this can make data analysis easier. Additional to this criterion, there is another related to two characteristic times more. These are the momentum relaxation time (τ_{MR}) and hydrodynamic time scale (τ_{hyd}) [3].

$$\tau_{MR} = \frac{(\rho_p - \rho_{liq})R_p^2}{\eta_{liq}} \quad (62)$$

$$\tau_{hyd} = \frac{d_p^2 \rho_{liq}}{\eta_{liq}} \quad (63)$$

Where, $(\rho_p - \rho_{liq})$ is the difference between the density of the polymeric dilution and the liquid solvent $\approx 0,1$ g/ml. R_p is the radius of the polymeric particles, in case of HA ≈ 200 nm. d_p is comparable to δ (penetration depth). According to section 4.2, the penetration depth is 178nm for a quartz crystal at 10MHz in contact with water. Solving the previous equations, the characteristics times are: ($\tau_{MR} = 4$ ps) and ($\tau_{hyd} = 31.9$ ns). These results are obtained considering water as liquid solvent and HA as polymer. The criterion specifies that both times must be smaller than the period of oscillation (τ_{osc})[3].

Now, considering the fundamental frequency of the crystals is 10MHz, the oscillation time ($\tau_{osc} = 1/F_{osc} = 100ns$). Considering HA dilutions in water as colloid dispersions and based on the previous calculus, working at 10MHz for characterizing HA dilutions is valid in principle.

4.8. Steps for characterizing the Quartz Sensor

For an adequate evaluation of the sensor response, it is necessary to define the parameters to measure, which depends of the application and the electronic interface used to acquire them. There are cases in which it is necessary a full characterization and there are other cases in which it is necessary to obtain only some key parameters. In the first cases, probably, an impedance analyzer is needed, and in the other cases, it is sufficient with a resonator circuit to know the behavior of the system.

According to the information at the beginning of this chapter, the typical parameters needed to characterize the behavior of the crystal with or without sample are the series resonance frequency (F_s), the half band half width (Γ) and the motional resistance (R_s) (Figure 1). F_s , Γ and R_s are obtained in experimental way using an acquisition system. No all acquisition systems provide all parameters. In most cases, only the resonance frequency is measured. The parameters of the equivalent electric circuit (BVD) are optional too. These can be calculated to simulate the circuit and compare the results with the experimental measures. Some values are given by the supplier of the crystal. For instance: the thickness of the crystal, the fundamental resonance frequency and its static capacitance C_0 .

Knowing the parameters of measuring, the first step is to place the crystal inside its holder and get the reference parameters (F_s , Γ and R_s) for a crystal without sample using the acquisition system.

Once the reference parameters have been obtained, the crystal cannot be manipulated until the measure of the sample has been done because the reference can change and it would not be useful.

The second step is to deposit the sample on the electrode of the crystal while the acquisition system is capturing data. The deposition can be in static or dynamic mode. In static mode, the deposition system can be a micropipette used to put the sample over the crystal directly. In dynamic mode, there is a fluidic system connected to the crystal holder in order to move and deposit the sample over the crystal. Normally, the quantity deposited is around some tens of microliters. For mass detection (proteins, DNA, nanoparticles, etc), it is necessary the dynamic mode because these kinds of samples have specific times of interaction. Moreover, the contamination problem is more critic in these applications and the fluidic system provides an environment protected. In characterization of fluids the static mode is acceptable.

The new parameters (F_s' , Γ' and R_s') measured while the sample was deposited, are compared with the reference values using the equations 1, 2 and 3 and ΔF , $\Delta \Gamma$ and ΔR are obtained. Depending of the nature of the sample, the variable of interest can be different. For rigid samples or Newtonian fluids, ΔF is more relevant according to previous sections. For viscoelastic samples (soft matter), it is necessary ΔF and $\Delta \Gamma$ in order to obtain an adequate characterization [48]. In particular, the method proposed in Chapter III – The Proposed Biomedical Application: Potential diagnosis of arthritic disease uses the three parameters.

In many cases, the biosensing applications need a liquid environment to deposit the biological mass layer; for instance: culture cells or protein detection in a biological fluid. For these cases,

the effect in the crystal response is accumulative [48]. Therefore, it is necessary to characterize the liquid without the biological mass layer deposited. After, the mass is put in contact with the crystal through the liquid and it is necessary to measure again. The values (F_s' , Γ' and R_s') obtained for the liquid are used as reference for the biological sample deposited. A fluidic system is very useful to control this process.

For these applications, it is recommendable an acquisition system capable to register the variables of interest in real time. The measure can take a couple minutes until several hours depending of the application.

In applications of mass detection, the sensitivity of the acquisition system is very important because the change of the frequency related to mass deposition is only a few hertz. In characterization of liquids, the change in frequency due to liquid deposition is usually, the hundreds of hertz.

4.9. Acquisition systems for piezoelectric sensors

There are some principal techniques used for the characterization of the crystal response: impedance analysis, impulse excitation, oscillators and lock-in techniques.

4.9.1. Impedance analyzer

The Impedance analyzer equipment measures the impedance or admittance of the quartz crystal in a wide range of frequencies for a complete characterization in the frequency domain. The sweeps must be done close to resonance frequency in order to obtain the characteristic curves (Figure 1) and the parameters of interest. Nowadays, there are many equipment capable to obtain a complete impedance characterization in a wide frequency spectrum, i.e.: the precision Impedance Analyzer Agilent 4294A with a range of 40 Hz to 110 MHz.

Figure 15. Precision Impedance Analyzer (reproduced from www.keysight.com/)

The use of these equipment is the easier way to obtain the frequency response of the crystal because it is not necessary any other external circuit connected to the crystal. In addition, the impedance analyzer is capable to obtain the resistance and reactance or the conductance and susceptance with a good precision. Knowing the conductance curve, it is possible to obtain the series resonance frequency F_s , R_s and the quality factor Q . Also, it is capable to compensate the external influences over the crystal by an adequate calibration of the instrument.

However, for biosensor applications, an impedance analyzer has two important disadvantages: its high price and it is not a portable equipment. In any case, the impedance analyzer is very useful as a first approximation to understand the behavior of the crystal.

4.9.2. Adapted equipment of sweep frequency

Using a similar principle to the impedance analyzer, several electronic circuits have been designed to obtain the spectrum of impedance or admittance of the crystal in a range of frequencies close to resonance [37,79–81]. These electronic circuits can obtain the series resonance frequency (f_s) and the half band half width (Γ) for each measure cycle in a few seconds. Typically, the electronic circuit is configured for a specific range of frequencies, depending on the fundamental frequency of crystal; for example: if the fundamental frequency of resonance of crystal is 10MHz, the electronic circuit is designed to perform a frequency sweep close to 10MHz.

4.9.2.1. The Calvo circuit

In the circuit proposed by Calvo et al. (Figure 16) a capacitor is connected in series to the crystal. A sinusoidal signal is applied between the external capacitor and the grounded crystal electrode [81]. In this study, the authors perform several frequency sweeps close to the resonant frequency of the crystal, they obtain the average values of the input and output voltages using an analog/digital converter and a computer.

Figure 16. Sensor schematic for Calvo technique (Adapted from [81])

Figure 17. shows the resultant spectrum function $|V_o/V_i|$ (Reproduced from [81])

The transfer function spectrum is composed by 100 frequencies points around the natural resonant frequency of the crystal. The spectrum is built in 0.25 s (real-time evaluation) [81].

The resultant equation for the transfer function according to Calvo circuit is:

$$\left| \frac{U_o}{U_i} \right| = \frac{\sqrt{(\omega L - 1/\omega C)^2 + R^2}}{\sqrt{\left(\omega L - 1/\omega C + \omega L C_0 / C_{ext} - C_0 / \omega C C_{ext} - 1/\omega C_{ext} \right)^2 + \left(R + R C_0 / C_{ext} \right)^2}} \quad (64)$$

The equation 60 is used to find the electrical parameters in the circuit. The experimental that best fit to the experimental results. The values are obtained by a non-linear fitting using appropriate conditions [47,81].

Using this method, the authors have obtained also the electrical equivalent circuit elements shown in Figure 16. The results are shown in Table 1.

	QUARTZ	WATER	HYDROGEL IN AIR
X_{LF}/Ω	4.7×10^5	214	364
L/MH	7.5	3.5×10^{-3}	7.45×10^{-3}
R/Ω	90	214	0

Table 1. Typical values of the quartz crystal equivalent circuit components (adapted from [81])

4.9.3. Impulse excitation and decay method

The impulse excitation method was introduced by Rodahl and coworkers [82–84]. This method consists of exciting the quartz crystal with an electric impulse signal between its electrodes. If the excitation is an ideal impulse, the quartz resonator will oscillate influenced only by its acoustic properties (mechanical damping). These oscillations will damp the amplitude due to the resistance component in the crystal. The effects of the external electronic circuit are negligible [55]. Which is the principal advantage of this method because the oscillation frequency depends only on the motional elements.

The company Q-Sense has patented this method as “The QCM with dissipation monitoring (QCM-D)” [85]. The QCM-D method consists in obtaining the dissipation factor (D) by sensing the decay of the crystal oscillations after a short excitation pulse close to the resonant frequency (F0). The decay rate is proportional to the energy dissipation of the oscillator [86,87].

With the aim to sense the decay time of the oscillation, the oscillatory voltage between the electrodes is obtained and adjusted after the driving power is switched off. In this way, the signal in the quartz decays close to the series resonant mode [87,88].

The amplitude decay rate depends on the properties of the oscillator and the sample under study [83,86,87,89]. To obtain the output frequency (F), the signal sensed with a frequency given by the resonant frequency of the quartz crystal (F0) is mixed with a reference frequency (Fref) and filtered with a low pass band filter. This procedure gives an output frequency (F) related to the difference between F0 and Fref ($F = F0 - Fref$) [86–88].

The response of the oscillator can be expressed as:

$$F(t) = A_0 e^{t/\tau} \sin(2\pi ft + \theta) \quad (65)$$

Where: A_0 is the amplitude at $t=0$, τ is the decay time constant and θ is the phase.

The frequency can be determinate by:

$$f \approx F_s \sqrt{1 - 1/4Q^2}; \quad (66)$$

$$Q = \frac{\omega_s L}{R}; \quad (67)$$

Where F_s is the series resonance frequency of the crystal and Q is the quality factor.

In addition, the dissipation parameter is inversely proportional to τ :

$$D = \frac{1}{Q} = \frac{1}{\pi F t} = \frac{E_{dis}}{2\pi E_{sto}} \quad (68)$$

Where Q is the quality factor. The parameter D can be also expressed as the Energy dissipated (E_{dis}) during one oscillation period over the Energy stored (E_{sto}) in the oscillating system [86,87]. The parameter D is dimensionless. The following figure explains the technique QCM-D behavior:

Figure 18. Impulse excitation QCM-D method. (Adapted from [90]).

In QCM-D method, the voltage between the electrodes is intermittently switched off and the decay time of the oscillation is observed. According to Figure 18, the resonant frequency $f=F_s$ and the energy dissipation $D = 2\Gamma/F_s$ are extracted from the decay curve. The blue curve corresponds to a crystal oscillating in air. The red curve corresponds to a crystal in contact with sample generating dissipation [90].

The resolution reached with this technique is around ± 0.1 Hz for the frequency and 1×10^{-7} for the dissipation factor [86].

Furthermore, the QCM-D technique permits to find F_s and D parameters at different overtones ($n=1,3,5,\dots$) of a fundamental resonant frequency [86].

QCM-D technique has been used widely in different studies, i.e: cellular characterization [25,26,91–93], protein detection [27,94–98] and DNA detection [99–104], and viscosity analysis of different substances [37,46,67,100,105–108].

Immobilization of DNA and Proteins can be accurately measured by tracking changes in frequency and fitting these data to the Sauerbrey equation. However, in the case of samples containing water, as in protein, cellular studies or viscosity analysis, the factor D is a fundamental value to obtain a full characterization of the viscoelastic layer structure absorbed [86,91,93].

According to M. Dixon[86], the size and flexibility of the layer deposited on the crystal electrode is directly proportional to the factor D. Furthermore, plotting ΔF vs ΔD permits to obtain information about the conformation of the absorbed material and quantify variables related to viscoelastic properties (shear viscosity and storage modulus) [86].

QCM-D technology was commercialized by Q-Sense in 1996. Nowadays, there are many different versions of QCM-D instruments available from Q-Sense; a single channel version, four channel version, combined technique with microscopy, electrochemistry or ellipsometry [85].

4.9.4. Oscillators

The principal advantages of the oscillators in QCR applications are continuous monitoring, integration capability and low cost of fabrication. However, when the application implies the crystal in contact with liquid or heavy loaded sensor, the oscillators are not a good idea because the Q factor of the resonator is strongly reduced; for instance, for a crystal at 10MHz its Q factor is reduced from 80.000 to 3.000 in contact with water [47]. Moreover, the losses due to external variables as the temperature and electric noise have more effect over the crystal behavior.

Also, the oscillator system does not obtain the passive form the resonance frequency of the crystal. In other words, the frequency at which the crystal oscillator is driven depends on the resonator phase condition in the loop and the resonator phase condition depends on the working phase of the rest of the components of the circuit [47]. An acceptable solution for this problem is make a good selection of components and appropriate configuration of oscillator to maintain the phase as constant as possible in the frequency range of interest.

With the aim of generating stable oscillations, the oscillator circuit must excite the quartz crystal with a frequency where a sharp phase slope occurs [55]. This condition is fulfilled at the characteristic frequency (F_r). Therefore, using the oscillators method, the quartz crystal does not oscillate at series resonance frequency because $F_r \neq F_s$. For crystals in contact with sample, this problem increases. In order to correct this phenomenon, the stray capacitance C_0 should be externally compensated [47,50,55,57,109,110].

According to the equivalent circuit model BVD, the phase for a quartz crystal is defined as:

$$\varphi = \frac{Im(Z)}{Re(Z)}$$

Where Z is the equivalent impedance of the crystal. When the capacitance C_0 is compensated, $Z = Z_m$. Where Z_m is the equivalent impedance for the series arm in the model (motional impedance).[32,42,48,52,55,57,58,111–113] In this case, the phase can be defined as:

$$\varphi = \frac{Im(Z_m)}{Re(Z_m)} = \frac{\omega L_s - 1/\omega C_s}{R_s}$$

After C_0 compensation, the conductance value G is max ($Re[Z_m] \rightarrow \max$), the susceptance value B is zero ($Im[Z_m] \rightarrow 0$) and the phase $\varphi=0$ at series resonant frequency F_s . In other words, making the C_0 compensation, the crystal vibrates at F_s . According to Wolfbeis [55], the difference in the resonant frequency F_r before the C_0 compensation and after the C_0 compensation for a 10MHz crystal in contact with water is around 144Hz.

In the oscillators methods, there are other variables called A and k , related to the open loop gain and feedback factor respectively. Using these variables, the electronic circuit must be guarantee a stable oscillation compensating the sample damping. Some studies propose an automatic control for these variables [109,114–116].

There are several designs of oscillator to work with liquid samples; for instance: emitter coupled oscillators [117–120], ACC oscillator [57,121], lever oscillator [114], active bridge oscillator [115] and Bridge symmetric crystal oscillator [116].

4.9.5. Lock-In Techniques

The circuit proposed by Nakamoto[42] is composed by a voltage controller crystal oscillator (VCXO) and the RF voltage supply as a system of amplitude gain. Both systems create the variable input RF signal to the crystal (Figure 19).

Figure 19. Circuit block diagram of Nakamoto (Adapted from [42]).

As RF current detector, Nakamoto and coworkers have used a toroidal core transformer (I-U Converter) [42]. Both signals, the RF voltage supply and the current signal obtained are supplied to a double balanced mixer (DBM) which output is the product of these signals.

Figure 20. Circuit block diagram of Nakamoto (Adapted from [42]).

According to Figure 20, the output signal has two components: DC and 2ω component. The 2ω component is eliminated by a low pass filter (LPF). The DC signal is proportional to the real part of the crystal admittance [42].

The same method has been used by other authors as Jakoby [47,109]. Jakoby et al. include a similar scheme of the Figure 20 in another loop indicated in Figure 21.

Figure 21. Sensor schematic for Jakoby technique (Adapted from [47]).

In the Figure 21, the second integrator loop permits to modulate in frequency the feedback signal and add it to the input signal. The output signal from integrator determines the central frequency of the sweep. The amplitude of the input signal controls the sweep range. As result, the circuit detects the peak of conductance curve automatically.

5. Multichannel Quartz Crystal Resonators

Multichannel quartz crystal resonator is a set of several resonators in the same sensor system. This system works as a full sensor platform to analyze the same sample with different characteristics. For example, the platform permits to analyze the same sample to different overtones simultaneously or also each individual electrode can be functionalized with a specific detection layer in order to identify different compounds in the same sample. In particular, multichannel quartz crystal microbalance (MQCM) is a set of some microbalances on a single quartz substrate. Miniaturizing QCMs is necessary for developing MQCMs, as large arrays must be constructed. QCM miniaturization leads to additional benefits including lower cost, less sample/reagent consumption, faster sensing response and shorter diagnostic time. However, MQCMs still should overcome the challenge of avoiding interferences.

Conventional MQCM consist of an array of electrode pairs deposited on both sides of an AT cut quartz crystal usually of a thickness of few hundreds of microns. Acoustic wave induced by one QCM will propagate laterally and may interfere with QCM at the vicinity causing acoustic interference.

In large arrays space between electrodes must be small, which contributes to increasing the interferences, mainly if the QCMs are identical and operate at the same resonance frequency. Besides of spaces between electrodes, acoustic interference depends on MQCM device parameters such as geometry, dimensions and thickness of each electrode, mechanical and piezoelectric properties of quartz substrate and electrode materials.

Usually, impedance measurements of the two QCMs under exchanged external load may be used to experimentally study interference between two contiguous QCMs and an electrical equivalent circuit, such as Butterworth–Van-Dyke (BVD) model and transmission line representation, is used to model and describe the response. These models show a simplified, one-dimensional, MQCM configuration as the vibration profile is assumed to be uniform throughout the electrode surface. Modeling results are generally valid near the resonance frequency. However, it neglects important features such as coupling modes and energy trapping of electrode layer.

In addition to acoustic interference, other problems of conventional MQCM include spurious vibration, limited sensitivity and limited size of electrodes. Spurious vibration occurs because of coupling between thickness shear (TS) and other modes including thickness flexural (TF), face shear (FS), thickness twist (TT) and higher order modes [122].

In order to improve the MQCM behavior, several design strategies could be implemented: to use different electrode sizes to avoid strong interferences, to increase the electro size for improving electrode trapping (although this could bring higher order modes and introduce higher order frequency interference) and to increase the electrode separation. Other strategy consists in modifying the structure of the quartz crystal in order to obtain higher frequency. The following modifications are the more typical: Mesa-Shape and convex shape.

Figure 22. mesa-shaped structures: (a) plano-mesa (b) plano-inverted- mesa (c) bi-mesa and (d) bi-inverted-mesa. (Adapted from [122])

Figure 23. convex-shaped structures: (a) plano-convex and (b) bi-convex. (Adapted from [122])

In this work is proposed the prototype as a multichannel QCR with 4 crystals organized in an array to analyze the same sample with different conditions. For instance: crystals with different resonant frequency or functionalized with different detection layers. The monolithic multichannel crystals are very expensive, for this reason, these are not used in this work.

6. Chapter II – The Biosensor Design

According to CHAPTER 1 - Fundaments and QCR Theory, the best systems for characterizing the behavior of the crystal are which stimulate the crystal and obtain the variables of interest in passive way. For instance: the admittance analyzer and impulse decay methods

In addition, it is wanted that the biosensor supplies as much information as possible about the behavior of the crystal. The portability and low cost are other desirable characteristics.

In this chapter, the design of a QCR sensor based on Nakamoto circuit[42] and the sweep frequency technique is proposed.

There were two versions until the final prototype. The first one was designed and implemented with the aim of evaluating the general circuit for obtaining the voltage, the current and the susceptance of the crystal. In this way, it is possible to obtain a full characterization of the crystal behavior with and without sample.

The second design is a better adaptation of the first one; this version has been implemented for having more portability, reducing the weight and improving the time of measure. The final version includes these enhancements along with an adequate crystal holder. Moreover, the final version has the possibility of testing more than one crystal at the same cycle of measure. This version also includes a software designed in Labview for the auto calibration of the biosensor with the aim of obtaining the best signal-noise relation for each data curve.

6.1. Crystals and Holders

Quartz crystals with fundamental resonant frequency of 10MHz and gold electrodes have been used. This crystal was supplied by Instrumatic Española, S.A. (Figure 24)

Figure 24. Crystals used in experimental tests

The fundamental frequency in both crystal is 10MHz. In consequence, the thickness is the same. The diameter of the crystal with gold electrodes is 25mm and the gold electrode diameter is 5mm. The diameter of the crystal with aluminum electrodes is 8mm and its electrode diameter is 4mm.

Two holders have been done due to different diameter of the crystals but, both have the same design. Two o-ring are used to hold the crystal plate. These holders are commonly named “static cells” due to absence of fluidic connections.

Figure 25. Holder developed (Static Cell)

Figure 25 shows the holder used. The two parts are adjusted using four screw. On top of the holder, the liquid sample is deposited using a pipette.

Figure 26. Holder developed (Fluidic Cell in parts)

Figure 27. Holder developed (Fluidic Cell assembled)

Figure 26 and Figure 27 show the fluidic cell designed to deposit the fluid sample over the crystal electrode using a pressure pump. This technique permits to obtain measures about the behavior of the crystal and depositing the samples without the necessity of manipulating the crystal and maintaining a constant fluid sample. The sealing system is based on magnets, the magnets permit a very reliable sealing and easy way to open and close the cell.

6.2. Proposed Design

Based on section 4.9, the electronic acquisition system designed with the purpose of obtaining the response of the crystal with and without samples was a variation of the Nakamoto and Kobayashi proposal [42]. According to Figure 28, the quartz crystal is placed in series with a toroidal-core transformer used as current sensor. In fact, the current across the crystal is detected using this toroidal transformer. The secondary coil of the transformer gives a voltage proportional to the current through the crystal. The voltage is shifted 90° respect to the original current signal. The number of turns is the same for primary and secondary coils (1:1). The voltage signal (V2) related to the current through the crystal (I1) and the voltage used to stimulate the crystal (V1), are multiplied according to Figure 28. The resulting signal is filtered to obtain the DC component. This DC component is related to the susceptance of quartz crystal. In addition, V1 and V2 signals are rectified to obtain the peak values for each frequency. In section 6.5.1 the first design is explained in detail.

With the aim of understanding the behavior of the quartz crystal and validating the proposed design, simulations of the geometric and equivalent electric models have been done.

Figure 28. Proposed design for acquisition circuit.

6.3. Simulations

In principle, there are two possible models whose simulations permit to explain the behavior of the crystal. The geometric model and the equivalent electric model BVD. The first one recreates the crystal as a quartz plate with two electrodes of gold permitting of analyzing the mechanical vibration of the crystal and its distribution over the surface. Moreover, it is possible obtain information about the fundamental frequency and the conductance and susceptance curves. However, the results obtained using the geometric model does not consider the losses related to external components as electronic circuits needed to obtain the response.

6.3.1. Geometric Model

COMSOL Multiphysics was used to simulate the geometric model in order to understand the behavior of the crystal with and without sample. Two different simulation studies were done. The first one is a eigenfrequency study done for determining the resonance frequency of the model. The results are shown in Figure 30. The second one is a frequency domain study done with the intention of determining the behavior of the crystal admittance. These results are shown in Figure 31.

The quartz crystal has been modeled with two electrodes, the crystal diameter is 13.66mm, the electrode diameter is 5.1mm and the crystal Thickness is 167um (fundamental resonant frequency 10MHz). Since the AT cut Quartz Crystal forms 35.35 degrees with the Z-axis [123], the application of a voltage between the electrodes generates a shear deformation of the crystal. When the voltage applied is AC and with frequency close to the fundamental resonant frequency of the crystal, the shear deformation is periodic (periodic vibration) arising its maximum amplitude at resonance frequency. According to CHAPTER 1 - Fundaments and QCR Theory, the frequency of this vibration is sensitive to the contact with a deposited mass or fluid.

With the aim of modeling the geometry of the crystal, it is indispensable considering the relationship between the electrodes thickness (<100nm) and the crystal thickness (167um). For a QCR's, this relation is four magnitude orders. This result implies to make a very fine mesh to obtain good results, but more computational recourses are needed [124]. Therefore, it is better do not create the electrodes with thickness into the geometry and assume they have the same thickness as the total crystal area but with a different equivalent density using the following equation:

$$\mathbf{d}_{\text{electrodes}} = \mathbf{d}_q \times \left(1 + \left(\frac{\mathbf{d}_{\text{au}} \times \mathbf{h}_{\text{au}}}{\mathbf{d}_q \times \mathbf{h}_q} \right) \right)^2 \quad (69)$$

Where, $d_{\text{electrodes}}$ is the equivalent density of electrodes, d_q is the density of quartz crystal (2651 kg/m³), d_{au} is the density of gold (1930 kg/m³), h_q is the crystal thickness, h_{au} is the electrode thickness. Solving the equation, the equivalent density of the zone of electrodes is 2674 kg/m³ [124].

The applied voltage was 1V and a damping coefficient of 2% was used [123]. Considering the QCR is a Thickness Shear Mode (TSM) sensor, the mesh in the thickness direction is the principal variable to obtain good simulation accuracy. The Figure 29 shows the mesh of the crystal. The fundamental frequency value was stable when the number of mesh layers in the thickness was more than 18 [125].

Figure 29. Mesh of crystal geometry in COMSOL Multiphysics. The enlarged image corresponds to the mesh in thickness axis

According to Figure 29, the fluid is modeled like a cylinder (red cylinder) over the electrode of the crystal and it is meshed at the same way. The dimensions of the red cylinder are related to the quantity of the volume deposited on the crystal.

For the geometric model, two complementary simulations were done. The first one is a eigenfrequency study done for determining the resonance frequency of the model. The results are shown in Figure 30. The second one is a frequency domain study done with the intention of determining the behavior of the crystal admittance. These results are shown in

Figure 30 shows the total displacement in X axis of bare crystal (left) and with liquid sample (right). In both cases, the more vibration is in electrode center and decreases it with distance to periphery. In consequence, the more sensibility is in the center of electrode.

Figure 30. Multiphysics simulation in COMSOL

The total displacement in X axis (vibration axis) at resonance frequency for quartz crystal. As expected, the major vibration is in electrode center and decreases it with distance to periphery.

According to Figure 31, the resonance frequency for bare crystal is $f_s=9.850700$ MHz. In case of the crystal in contact with water, the resonance frequency is $F_s=9.848645 \times 10^6 + 1835.50i$. Using the equation (45), $f_s=9.848645$ MHz and $\Gamma=1835.5$ Hz.

Figure 31. Results for Multiphysics simulation – Conductance and Susceptance response

Figure 31 shows the conductance and susceptance graphs obtained in a geometric simulation. The results of the principal variables are shown in Table 2.

	Fs (Hz)	Γ (Hz)	Rm (Ω)
Air	9850700	2.3	0.098
Water	9848645	1835	330

Table 2. Results for Multiphysics simulation in COMSOL

According to Table 2, $\Delta f_s = 2055$, $\Delta \Gamma = 1832,7$ Hz and $\Delta R_s = 3367$ for a crystal in contact with water.

Using the Kanazawa equation 45, it is possible to obtain the value of the viscosity of the sample. The water viscosity = $1.03 \text{ mPa}\cdot\text{s}$ where the water density = 1000 kg/m^3 .

The motional resistance (R_m) is also interesting because is an indicative value of the “vibration force of the crystal”. According to Figure 31 and Table 2, the contact with water attenuates in two magnitude orders the vibration of the crystal. In other words, the losses in the crystal increase two orders of magnitude.

6.3.2. Equivalent Electric Model (BVD)

The electric model library in COMSOL was used for modeling the electric BVD model for quartz crystal. Solving equations 6, 7, 27, 35, 36, 37, 39, 40 for a 10MHz crystal with thickness of $167\mu\text{m}$ in contact with water ($\rho=1000\text{Kg/m}^3$ and $\eta=1\text{mPa}\cdot\text{s}$). The following values are obtained:

- $F_s = 9984729 \text{ Hz}$
- $C_q = 3.159 \times 10^{-14} \text{ F}$
- $L_q = 8.043 \times 10^{-3} \text{ H}$
- $R_q = 0.378 \Omega$
- $C_0 = 4.88 \times 10^{-12} \text{ F}$
- $L_s = 3.254 \times 10^{-6} \text{ H}$
- $R_s = 204.140 \Omega$

In addition, there is other inductance called (L_T) due to a toroidal core transformer in series to the quartz crystal.

$$L_T = N^2 * A_L; \quad (70)$$

Where A_L is the inductance factor and N is the turns number in the coil. According to the datasheet of the core transformer, the material of the core is N30 and $A_L = 2200$ nH. In consequence, the total $L_T = 55$ uH. The function of the transformer is explained in section 6.5.

The transformer influence in the total load (crystal + sample + transformer) is negligible because the transformer is in both instances, for a crystal undamped and in contact with samples. In other words, the frequency shift effect due to the transformer inductance can be consider as a fixed offset in the measures.

The equations 7 and 8 were used for calculating the resonance frequency and the half band half width using equivalent electrical parameters. For measures with water as sample, L_q and R_q are changed by (L_q+L_s) and (R_q+R_s) respectively, according to Kirchhoff laws [126].

Figure 32. Equivalent electric circuit BVD used in Simulations

Figure 32 shows the equivalent electric circuit BVD used to simulate the behavior of the quartz crystal inside the circuit net. R_{in} and L_T represent the input resistance of the signal IN source and transformer inductance respectively (external circuit). R_{in} for the first version of the prototype was around 8 ohms, for improved versions R_{in} decreased until 5 ohms. In the section 6.8, the theoretical value of C_0 and R_q are changed by C_0^* and R_q^* respectively. These values were obtained experimentally. The BVD model and the simulation was performed again with these modified parameters to compare both results.

Figure 33. Results for BVD model simulation – Admittance response

The conductance and susceptance curves obtained using COMSOL for simulating the schematic shown in Figure 32 are shown in Figure 33. Undamped crystal (Figure 33-left), crystal in contact with water (Figure 33-right). The results of the principal variables are shown in Table 3.

	Fs (Hz)	Γs (Hz)	Rs (Ω)	Fm (Hz)	Γm (Hz)	Rm (Ω)	Fr (Hz)	Γr (Hz)
Air	9984543	4	0.38	9984543	7	0.38	9984543	4
Water	9982524	2023	204,52	9982398	3942	203.74	9982524	2023
Delta	-2019	2019	538.2	2145	3935	536.16	-2019	2019

Table 3. Results for BVD Model using COMSOL.

Table 3 shows the results obtained for the model BVD using COMSOL. Sub-index (s) is related to conductance values, Sub-index (m) is related to admittance values and Sub-index (r) is related to susceptance values.

According to Table 3, $\Delta f_s = 2019$ and $\Delta \Gamma_s = 2019$ Hz for a crystal in contact with water. The analysis for susceptance values provides the same results: $\Delta f_r = 2019$ and $\Delta \Gamma_r = 2019$ Hz. These results are in accordance with the Newtonian properties of the water. According to Kanazawa equation (51), $\Delta f_s = \Delta \Gamma_s$ for Newtonian fluids. According to COMSOL simulation, $\Delta f_m = 2145$ and $\Delta \Gamma_m = 3935$ Hz much more in relation to conductance or susceptance. In consequence, the admittance parameter is not convenient to obtain the properties of the sample.

Using the Kanazawa equation (46), it is possible to obtain the value of the viscosity of the sample, in the simulation case, water. The water viscosity = 0,95 mPa·s where the water density = 1000 kg/m³.

In addition to COMSOL simulations, the equivalent circuit shown in Figure 32 has been also solved using Kirchhoff laws. The conductance and susceptance expressions were used instead of resistance and reactance ones because the values of interest are directly related with the changes in the conductance and susceptance curves.

$$Z_{total} = (R_{in} + j\omega L_T) + \left(R_q + R_s + j\omega L_q + j\omega L_s + \frac{1}{j\omega C_q} \right) \parallel \frac{1}{j\omega C_0} \quad (71)$$

Considering just the serial branch in the circuit and its equivalent admittance:

$$Y_{Serie} = \frac{1}{R_q + R_s + j\omega \left(L_q + L_s - \frac{1}{\omega^2 C_q} \right)}$$

$$Y_{Serie} = \frac{R_q + R_s - j\omega \left(L_q + L_s - \frac{1}{\omega^2 C_q} \right)}{\left(R_q + R_s \right)^2 + \left(\omega \left(L_q + L_s - \frac{1}{\omega^2 C_q} \right) \right)^2}$$

$$G_{Serie} = \frac{(R_q + R_s)}{\left(R_q + R_s \right)^2 + \left(\omega \left(L_q + L_s - \frac{1}{\omega^2 C_q} \right) \right)^2}$$

$$B_{Serie} = \frac{-\omega \left(L_q + L_s - 1/\omega^2 C_q \right)}{\left(R_q + R_s \right)^2 + \left(\omega \left(L_q + L_s - 1/\omega^2 C_q \right) \right)^2}$$

Now, considering the capacitance C_0 in parallel and its equivalent admittance:

$$B_{C0} = j\omega C_0$$

The conductance (G_{serie}) does not change due to the inclusion of the C_0 , just the susceptance

$$G_{Cristal} = G_{Serie}$$

$$B_{Cristal} = B_{Serie} + j\omega C_0$$

Now, transforming the external circuit impedance into its equivalent admittance:

$$Y_{in} = \frac{1}{R_{in} + j\omega L_T} = \frac{R_{in} - j\omega L_T}{R_{in}^2 + (\omega L_T)^2}$$

$$G_{in} = \frac{R_{in}}{R_{in}^2 + (\omega L_T)^2}$$

$$B_{in} = \frac{-\omega L_T}{R_{in}^2 + (\omega L_T)^2}$$

Considering the total admittance is:

$$Y_{total} = Y_{in} || Y_{crystal}$$

To obtain the total admittance Y_{total} , it is necessary to solve the mathematic process for two admittances Y_1 and Y_2 in parallel.

Where:

$$Y_{eq} = Y_1 || Y_2$$

$$Y_1 = G_1 + jB_1$$

$$Y_2 = G_2 + jB_2$$

The equivalent admittance is:

$$Y_{eq} = \frac{(G_1 G_2 - B_1 B_2) + j(G_1 B_2 + G_2 B_1)}{(G_1 + G_2) + j(B_1 + B_2)}$$

$$Y_{eq} = \frac{((G_1 G_2 - B_1 B_2) + j(G_1 B_2 + G_2 B_1)) * ((G_1 + G_2) - j(B_1 + B_2))}{(G_1 + G_2)^2 + (B_1 + B_2)^2}$$

$$G_{eq} = \frac{(G_1 G_2 - B_1 B_2) * (G_1 + G_2) + (G_1 B_2 + G_2 B_1) * (B_1 + B_2)}{(G_1 + G_2)^2 + (B_1 + B_2)^2}$$

$$B_{eq} = \frac{(G_1 B_2 + G_2 B_1) * (G_1 + G_2) - (G_1 G_2 - B_1 B_2) * (B_1 + B_2)}{(G_1 + G_2)^2 + (B_1 + B_2)^2}$$

Applying these results to Y_{Total} where:

$$G_1 = G_{in}$$

$$B_1 = B_{in}$$

$$G_2 = G_{crystal}$$

$$B_2 = B_{crystal}$$

The total conductance and susceptance functions are obtained. However, the equations are not shown in this document because are very extensive. The final equations have been introduced into an excel sheet and the total admittance was obtained for values close to resonant frequency.

In excel calculations, two different admittances (conductance and susceptance) have been obtained. The first one, named G_q and B_q , is related just to the mechanical branch in the equivalent circuit. In other words, these values do not consider C_0 and G_{in} . The second one, named G_{total} and B_{total} , considers C_0 and G_{in} influence.

Figure 34. Conductance for a crystal undamped. G_q curve was calculated without external influence. G_{total} was calculated considering G_{in} influence.

Figure 35. Susceptance for a crystal undamped. B_q curve was calculated without external influence. B_{total} was calculated considering the C_0 and B_{in} influence.

According to Figure 34 and Figure 35, external influences generate shift and attenuation in the admittance curves. The shift in the curves is generated by the inductance of the toroidal transformer and the attenuation is generated by resistive component of the external circuit. The shift and the attenuation in air is around 345 Hz and 32.6 times respectively.

Figure 36. Conductance (G_{total}), Susceptance (B_{total}) and Admittance (Y_{total}) for a crystal undamped.

Figure 36 shows the behavior of the crystal undamped (air) connected to external circuit. The attenuation generated by external influences, in particular by the external resistance, is compensated with an amplification system incorporated in the prototype.

Figure 37. Conductance for a crystal in contact with water. $G_{q+sample}$ curve was calculated without external circuit influence. G_{total} was calculated considering G_{in} influence.

Figure 38. Susceptance for a crystal in contact with water. $B_{q+sample}$ curve was calculated without the external circuit influence. B_{total} was calculated considering the C_0 and B_{in} influence.

According to Figure 37 and Figure 38, external influences generate the same shift in the conductance and susceptance curves for a crystal in contact with water. In other words, the shift caused by the inductance of the toroidal transformer is the same for an undamped crystal as for a crystal in contact with sample. Therefore, $\Delta f_{S,m,r}$ and $\Delta \Gamma_{S,m,r}$ are not altered by external influences.

Figure 39. Conductance (G_{total}), Susceptance (B_{total}) and Admittance (Y_{total}) for a crystal in contact with water.

Figure 39 shows the behavior of the crystal in contact with water connected to external circuit. The attenuation generated by external influences, in particular by the external resistance, is compensated with an amplification system incorporated in the prototype. Also, Figure 36 and Figure 39 confirm Δf_m is very similar to Δf_s and $\Delta \Gamma_m$ is bigger than $\Delta \Gamma_s$.

	Fs (Hz)	Γ_s (Hz)	Rs (Ω)	Fm (Hz)	Γ_m (Hz)	Rm (Ω)	Fr (Hz)	Γ_r (Hz)
Air	9984729	4	0.38	9984729	6	0.38	9984729	4
Water	9982710	2023	204.52	9982584	3942	203.72	9982710	2023
Delta	-2019	2019	538.2	2145	3936	536.16	-2019	2019

Table 4. Results for BVD Model without external influences using Kirchhoff laws.

	Fs (Hz)	Γ_s (Hz)	Rs (Ω)	Fm (Hz)	Γ_m (Hz)	Rm (Ω)	Fr (Hz)	Γ_r (Hz)
Air	9984384	126	12.37	9984384	218	12.37	9984384	125
Water	9982362	2153	212.18	9982225	4204	211.37	9982362	2144
Delta	-2022	2027	16.53	2159	3986	17.09	-2022	2019

Table 5. Results for BVD Model considering external influences and using Kirchhoff laws.

6.4. Simulations in Matlab

With the aim of contrasting results, Matlab 2015 was used for creating a simulation interface for quartz crystal resonators under different situations. In addition, to simulate in Matlab also reduces the computation time.

Moreover, using two different software for simulating the crystal behavior and obtaining similar results gives trust about the model and results.

Figure 40. User Interface for Matlab Simulations

Figure 40 shows the interface created using GUI tools in Matlab. The interface has as input data: the frequency range, the crystal electrode radius, density and viscosity of the liquid, the overtone of the input signal, the thickness crystal and the mass deposited over the crystal surface. With these data and using equations explained in Equivalent electrical model section the series resonant frequency (F_s), motional resistance (R_m) and half band half width ($Rho - \Gamma$) are obtained.

Figure 41. Matlab simulation for water deposition on quartz crystal surface

According to Matlab simulations, the variables of interest are:

	Fs (Hz)	Γ_s (Hz)	Rs (Ω)	Fm (Hz)	Γ_m (Hz)	Rm (Ω)	Fr (Hz)	Γ_r (Hz)
Air	9984543	5	0.38	9984543	7	0.38	9984543	5
Water	9982524	2024	204.5	9982398	3940	203.74	9982524	2024
Delta	-2019	2019	538.2	2145	3933	17.09	-2019	2019

Table 6. Results for BVD Model without external influences using Matlab.

According to Table 4, Table 5 and

Table 6, all simulation results (COMSOL simulations, results obtained using Kirchhoff laws and Matlab simulations) are really similar. These results confirm the model BVD is adequate to explain the behavior of the quartz crystal for a frequency range close to the resonant frequency. It is important to note, the analysis using conductance or susceptance curves generates the same response $f_s=f_r$, $\Gamma_s=\Gamma_r$ and $\Delta f_s = \Delta f_r$, $\Delta \Gamma_s= \Delta \Gamma_r$. However, in experimental results, there is a little frequency shift between the conductance and susceptance curves when the sample is deposited. In section 6.5.3 this phenomenon is explained in detail.

6.5. First Version of the Biosensor

In accordance with simulations explained in previous sections, the proposed design shown in Figure 28 is valid. Therefore, a first version of the biosensor was done. The proposed methodology for characterizing the behavior of QCR's consisting in two stages: the first stage involves the design and construction of a circuit for stimulating and acquiring the peak values of voltage, current and susceptance signals in the quartz crystal. The second stage involves processing data for obtaining the resonant frequency (f_s) value, the frequency at minimum impedance with zero phase (f_r) value, the half band half width (Γ) value and the maximum peak of conductance (G_s) related to the value of the motional resistance ($R_q=1/G_s$).

6.5.1. Stage 1 – Acquisition Data

The proposed circuit obtains voltage, current and susceptance values for several frequencies in a configurable bandwidth close to the crystal resonant frequency (f_c). This circuit is a variation of Nakamoto and Kobayashi proposal [42].

According to Figure 42, the prototype consists in a DAQ card for generating the sinusoidal signal (DAQ signal in Figure 43), the external circuit designed for stimulating and acquiring the signals of interest and the quartz crystal. Also, a software has been developed in Labview to control the prototype.

The software communicates the computer and the DAQ card with the purpose of obtaining voltage, current and susceptance signals of the external circuit and show them in the computer. In this first design, the power supply (+/- 5V) was provided by a laboratory source.

Figure 42. General Scheme of the prototype first version.

Figure 43. Circuit schematic of the first version of the electronic acquisition system. (reproduced from [37])

The circuit consists of an oscillator chip for generating a square signal of 10MHz of frequency; this output signal is filtered with a passive band-pass filter for obtaining the fundamental frequency of the signal. The filtered signal (sinusoidal wave) is multiplied with a sinusoidal DAQ output signal using a mixer (AD835AN – Analog Devices). The resulting signal is filtered and used to stimulate the quartz crystal. The frequency of the resulting signal is controlled changing the frequency of DAQ output signal. The multifunction DAQ used was a NI USB-6351 of National Instruments.

In accordance with designed schematic, the quartz crystal is placed in series with the toroidal-core transformer used as current sensor. The secondary coil of the transformer gives a voltage proportional to the current in the primary which is the crystal current. The voltage is shifted 90 degrees with respect to the original current signal. The number of turns is the same for primary and secondary coils (1:1). The voltage signal (V2) related to the current through the crystal (I1) and the voltage used to stimulate the crystal (V1), are multiplied in a second mixer AD835 according to Figure 43. The resulting signal is filtered using an active filter with LT1057 Amplifier to obtain the DC component.

The DC component is related to the susceptance of quartz crystal in accordance with the following equations:

$$V_1 = V_p \cos(\omega_1 t + \theta_1) \quad (72)$$

$$I_1 = I_p \cos(\omega_2 t + \theta_2) \quad (73)$$

$$V_2 = k I_p \cos(\omega_2 t + \theta_2 + \pi/2) \quad (74)$$

Where k is a proportional constant due to the relation between the coils in the toroidal transformer, ω_1 and ω_2 are the angular frequencies of the signals and θ_1 and θ_2 are the phases of the signals.

Based on the theory of the mixer of signals[127], the result of the multiplication can be written as:

$$V_1 V_2 = \frac{k}{2} V_p I_p \left[\cos((\omega_1 + \omega_2)t + \theta_1 + \theta_2 + \pi/2) + \cos((\omega_1 - \omega_2)t + \theta_1 - \theta_2 - \pi/2) \right] \quad (75)$$

As $\omega_1 = \omega_2$ because the signal of voltage on the crystal and the signal of current through the crystal have the same frequency, the equation (76) can be rewritten as:

$$V_1 V_2 = \frac{k}{2} V_p I_p [\cos(2\omega_1 t + \theta_1 + \theta_2 + \pi/2) + \cos(\theta_1 - \theta_2 - \pi/2)] \quad (76)$$

Then, by using a low pass filter, the high frequency component is removed:

$$V_1 V_2 = \frac{k}{2} V_p I_p [\cos(\theta_1 - \theta_2 - \pi/2)] \quad (77)$$

The following trigonometric identity is used to get the final equation:

$$\sin(A) = \cos(A - \pi/2) \quad (78)$$

$$V_1 V_2 = \frac{k}{2} V_p I_p [\sin(\theta_1 - \theta_2)] \quad (79)$$

Since the relation in the toroidal transformer is 1:1, the constant $k \approx 1$ so:

$$\text{Mixer output (DC component)} = \frac{V_p I_p}{2} [\sin(\theta_1 - \theta_2)] \approx |B| \quad (80)$$

It is important to note that the signal obtained is equivalent to the susceptance because the constant term ($V_p I_p / 2$) is modulated by the sine of the difference in the phase of the signals. In consequence, the signal obtained reproduces the behavior capacitive and inductive of the piezoelectric crystal in frequency. The zero-crossing occurs when the voltage on the crystal and the current through the crystal are in phase. When $0 < \theta_1 - \theta_2 < \pi$, the behavior of the crystal is inductive and $\pi < \theta_1 - \theta_2 < 2\pi$ the behavior of the crystal is capacitive.

In addition, a high-speed amplifier (AD8010ANZ – Analog Devices), is used together with a rectifier system and passive filters to obtain the peak of the signals of voltage (V_{out}) and current (I_{out}) with the DAQ acquisition system.

Voltage, current and susceptance signals are acquired in real time from a computer using the DAQ and Labview. Reading speed depends on the change in frequency sweep. A cycle of 5000 frequencies takes just 3 minutes.

6.5.2. Stage 2 – Processing Data

Voltage, current and susceptance data acquired in Labview, are subsequently exported to Matlab with the intention of performing the analysis of the crystal's behavior. The conductance magnitude curve $|G|$ is calculated using equation (77). According to Cassiède and coworkers, the baseline in conductance and susceptance must be removed to can fit them [48].

$$|G| = \sqrt{(|Y|^2 - |B|^2)} \quad (81)$$

The conductance and susceptance data are fitted using a summation of Gaussian functions and a summation of sine functions respectively. In both cases, adjustments are made with the least mean square error (RMSE) possible. The fitting is achieved using the Matlab Curve Fitting tool.

Summation of Gaussian functions:

$$G(f) = a_1 e^{-\left(\frac{f-b_1}{c_1}\right)^2} + a_2 e^{-\left(\frac{f-b_2}{c_2}\right)^2} + \dots + a_n e^{-\left(\frac{f-b_n}{c_n}\right)^2} \quad (82)$$

Summation of sine functions:

$$B(f) = a_1 \sin(b_1 * f + c_1) + a_2 \sin(b_2 * f + c_2) + \dots + a_n \sin(b_n * f + c_n) \quad (83)$$

The coefficients of the conductance and susceptance curves after fitting them are registered but not analyzed because the curves parameters are different for each quartz crystal and the coefficients do not give additional information. When the crystal is in contact with liquid, the coefficients also change because the morphology of the curves changes too. The number of terms in both functions can also change, depending on the quality factor of the quartz crystal and the liquid sample properties. However, it is important of adjusting the curves for minimizing the noise in acquired signals. The parameter used to select the appropriate fitting function for conductance and susceptance curves was the RMSE (Root Mean Squared Error).

Figure 44. Adjustment of the conductance and susceptance curves in Gaussian and sum of sine functions respectively. (reproduced from [37])

Figure 44 shows the fitting of the conductance and susceptance curves. In general, the fitting of the conductance curve is more accurate than the fitting of the susceptance curve. However, in the zone of interest (Frequency range between the maximum and minimum value of the susceptance curve), both fitted curves are very similar to the original data.

After adjusting the susceptance and conductance curves, the admittance phase is obtained as reference to the shift the susceptance across the frequency-axis. The admittance phase is obtained with the following equation:

$$\theta = \tan^{-1} \left(\frac{|B|}{|G|} \right) \quad (84)$$

The same acquisition and processing steps were realized for the undamped crystal and for the crystal in contact with water.

Figure 45 shows that the crossing between susceptance and admittance phase curves corresponds with the conductance peak for the undamped crystal. In other words, the series resonance frequency (F_s) and frequency at minimum impedance with zero phase (f_r) are the same for a crystal without sample (the vertical line drawn in black was set manually to show the phenomenon). This experimental result is in accordance with the simulations results.

Figure 45. Curves for undamped crystal. Magnitude of Conductance $|G|$ (green), Magnitude of Susceptance $|B|$ (blue), Admittance Phase curve (red). (reproduced from [37])

Figure 46. Curves for crystal in contact with water. Magnitude of Conductance $|G|$ (green), Magnitude of Susceptance $|B|$ (blue), Admittance Phase curve (red), First Derivative of $|G|$ curve (Turquoise). (reproduced from [37])

Figure 46 shows how the water sample shifts the susceptance curve to the right, so the crossing between the susceptance and the admittance phase does not match with the peak of the conductance. This phenomenon occurs because the water sample generates a phase shift which is usually negligible. In other words, the series resonance frequency (f_s) and the frequency at minimum impedance with zero phase (f_r) mismatch. To demonstrate this phenomenon, the first derivative of the conductance curve has been calculated and its zero-crossing point is compared with the zero crossing of the susceptance curve and admittance phase. Moreover, two vertical black lines were placed to graphically show the difference in the values at zero crossing.

An explanation related to this phenomenon is shown in detail in the following section.

6.5.3. Relative Permittivity measure with QCR

According to section 6.5.2, in experiment results is evident a shift between conductance and susceptance curve. In this section, this shift is related with a change in C_0 (capacitance in parallel to serial branch in Figure 32). At the same time, the change in C_0 is related with the sample permittivity. The tests were done with water milli-q. This effect was detailed by Rodahl et al [88].

According to Rodahl et al. and own observations, there is an additional capacitance value due to dielectric sample in contact with the crystal electrode; for instance: water milli-q. The Figure 47 shows the phenomenon.

Figure 47. Electric field lines into the quartz crystal resonator

In air, the origin of the static capacitance, C_0 , is mainly (but not entirely) due to the capacitor formed by the two electrodes and the quartz plate between them. In Figure 47 left, there is a small "spillover" of the electrical field, a so-called fringing field [88].

This fringing field causes a stray capacitance that contributes to the static capacitance C_0 of the QCM. The value of this additional capacitance depends mainly on the permittivity of the media which the field lines pass through; higher permittivity means larger equivalent capacitance. Air and quartz have relative permittivity constants of 1 and 4.55 respectively. When the crystal is oscillating without sample, the stray capacitance is relatively negligible because the medium has a low permittivity constant. However, when the crystal is in contact with a liquid with higher permittivity than air, for instance: water milli-q with a relative dielectric constant of 81 at 18°C, the stray capacitance is increased [88].

To calculate the increase in the equivalent C_0 , the relationship between the series resonance frequency (intercept of the first derivative of the conductance curve with the frequency axis) and the frequency at minimum impedance with zero phase (intercept of the susceptance curve with the frequency/horizontal axis) is used [128]. Figure 46 shows the phenomenon.

Using the following equations is possible to calculate the capacitive effect of the sample:

$$f_r = f_s \left(1 + \frac{1}{2QM} \right) \quad (85)$$

Where M is the crystal figure-of-merit and Q is the crystal quality factor:

$$M = \frac{1}{\omega_s R_q C_0^*} \quad (86)$$

$$Q = \frac{1}{\omega_s R_q C_q} \quad (87)$$

Solving the equations 85, 86 and 87 for C_0 when the crystal is in contact with water:

$$C_0^* = \frac{2\left(\frac{f_r}{f_s}-1\right)}{C_{q^*}(w_s R_q)^2} \quad (88)$$

$$C_s = C_0^* - C_0 \quad (89)$$

Equation 88 shows the total capacitance C_0^* . C_0^* is the sum of capacitance due to the electrodes and external cables (traditional C_0) and the change generated by water sample. To know the change in C_0^* , the component due to the electrodes shown in equation (27) is subtracted. The values with subscript (q) refer to the quartz crystal and capacitance value with subscript (s) refer to the sample. The section 6.10 shows the results for C_0^* .

6.6. Second Version of Biosensor

The second version of the Biosensor can be considered as an improved version of the first one. In the first version, has been validated the correct performance of the proposed circuit. In this version, the main objective was to improve the portability of the biosensor and the acquisition time of the signals from quartz crystal. The changes were focused in the acquisition system and the voltage supply system.

6.6.1. Improve Acquisition Data

The generation and acquisition system of the first version (DAQ – National Instruments) shown in Figure 42 was replaced by an arduino DUE card (Figure 48). This change represents a reduction around 1kg of weight in the prototype and 50% less in size.

In addition, this change improved the acquisition time of the voltage, current and susceptance signals from the electronic system shown in Figure 43. The ADC's of the arduino card were used for acquiring the three signals, voltage, current and susceptance respectively.

Figure 48. General Scheme of the prototype second version.

However, the delta of frequency in the sweep range lost resolution compared to acquisition system DAQ of National Instruments because the DAC into the arduino card was not fast enough.

In other words, the arduino card improved the acquisition of the signal but it worsened the generator system because decreased the number of frequencies into the sweep range. The solution at this problem is proposed in the final version.

Additional software in Labview was done to communicate the user interface and the arduino card. The communication protocol used was a serial synchronized UART using the VISA modules of Labview (Figure 49).

The new interface, made in Labview, has as user inputs: the range of frequencies to sweep, the step of frequency and the amplitude of the signal. The range of frequencies indicates the frequency window to make the sweep. By default, the window is 50KHz. The step of frequency indicates the change between frequencies evaluated. By default, the step of frequency is 10Hz. Both variables, the range of frequencies and the step of frequency define the number of frequencies evaluated inside the window. By default, the number of frequencies evaluated is 5000. Finally, the amplitude of the signal is the peak value of the sinusoidal signal that stimulate the crystal. This value is controlled by a amplification system.

In accordance with Figure 49, these data are concatenated into a frame data (pink blocks) and send them to arduino card through VISA protocol. VISA protocol permits to configure the number of bytes, the parity, stop byte, timeout and baud rate of the frame data.

Arduino card generates the low frequency sinusoidal signal (previous DAQ signal in Figure 43). Arduino card also gets the voltage, current and susceptance signal from circuit of Figure 43 and creates a new frame data with these signals. The new frame data is sent to Labview using the VISA block to reception. According to Figure 49, the data are decoded in a loop and each signal is filtered to obtain its shape without noise.

Figure 49. Labview software developed for communication with arduino card

6.6.2. Improve Processing Data

In this second version, a new software in Labview has been done to replace the processing data in Matlab. This software is capable to obtain all parameters of interest from the voltage, current and susceptance curves. The software gets the conductance curve using the voltage, current and susceptance data according to the equation (77) and shows the series resonance frequency (f_s)

obtained of the conductance curve (G). The frequency at zero phase (f_r) is obtained of the susceptance curve (B). The motional resistance (R_q) is also obtained from the conductance curve along with the half band half width (Γ).

This improvement in the software permits decrease the processing time of the data. The first version took between 10 and 20 min acquiring and processing one frequency sweep and gets the variables of interest in Matlab. The second version take 12 seconds acquiring, processing and gets the variables of interest in Labview interface. In case of characterize liquids, the acquiring and processing time is very important because if the system is very slow, the liquid sample can have significant changes in its volume and its behavior due to changes in temperature and external vibrations.

The Figure 50 shows the block diagram of the Labview software to processing data. After the voltage, current and susceptance signals are obtained from the frame data and filtered, the signals are converted to integer number arrays. Each number corresponds to one point in the conductance or susceptance graph. The frequency axis (horizontal axis) is also creates using the range of frequencies and step of the frequency value. Personalized functions have been created to obtain the values of interest.

The series resonance frequency (f_s) is obtained using the “Array Max & Min Function” block; this block is included into the “F Reso” function block. With the same block, the inverse value of the motional resistance R_q is obtained.

To get the frequency at zero phase (f_r) the susceptance curve is derivate. Due to the f_r happens at the zero-crossing point of the susceptance curve; the derivative signal of the susceptance in this point will be the minimum value of the signal. The value of the derivative signal of susceptance is minimum due to the slope in the susceptance curve is negative. The function derivative is included in the “deriva” function block in Figure 50.

Figure 50. Labview software developed for processing data

The conductance, susceptance, voltage and current signals obtained are shown in Figure 51.

The Figure 51 also shows the user interface where the necessary parameters (Frequency Min, Frequency Max, Delta of Frequency and Amplitude of the input signal) are entered by the user along with the variables of interest (F_s , F_r , $F_s - F_r$, Γ_l and Γ_r)

The additional change in C_0 commented in 6.5.2 is related to Delta $F_s - F_r$.

Figure 51. User Interface to show the results

6.6.3. Improve Supply System

The supply system in first version was a power differential supply of the laboratory. This system did not permit the portability of the prototype. Therefore, a new circuit with batteries and power adapter was designed to provide power to the general system. Two 9V batteries were used along with two voltage regulators (7805 to +5V and 7905 to -5V). In this way, the complete system takes minimal storage space, 10cm² of area.

According to Figure 42, the power supply gives energy to three systems: the DAQ of NI, the generator and the electronic system detailed in Figure 43. In Figure 48 can see that the generator system does not get power of the batteries because arduino card obtains its power supply of the computer. This represent one advantage because increases the operating time of the system. Using the battery system, the prototype got an operating time of three hours.

In addition, an AC power adapter (Figure 52) was designed to provide energy to the prototype in case the batteries are not present.

Figure 52. Adapter

Figure 53. Schematic for the adapter

The design of the adapter is based on a rectifier system with central tap as the shown in Figure 53

6.7. Final Version of Biosensor

The final version of the Biosensor includes a new DAC to improve the resolution in the frequency sweep. In particular, the frequency step decreases until 1Hz using the new DAC. AD9850 module is capable to create a sinusoidal signal between 1 to 40MHz with a frequency step of 1Hz. According to Figure 54, the new circuit design, the AD9850 module replaces the oscillator chip of 10MHz of frequency, the passive band-pass filter the first mixer (AD835AN) and the “DAQ signal” shown in Figure 43.

Figure 54. General Scheme of the prototype final version.

Figure 55. Scheme for communication with AD9850 DDS module and digital resistor

In accordance with Figure 55, the output signal of the AD9850 module is connected to an amplifier chip (OPA690IDR) with variable gain to control the amplitude of the signal. The control of gain is achieved from Labview interface using a digital controlled potentiometer (X9C102P). The output signal is connected to the toroidal core transformer in series to the quartz crystal.

The gain system controlled by Labview is very convenient because permits a calibration the system for obtaining the best relation signal/noise in the current, voltage and susceptance signals. Moreover, when aqueous sample is in contact with the crystal, the signals are attenuated in a factor of 10 approximately. In this case, the software permits to increase the gain changing the resistance value in the digital potentiometer. In this way, the losses in the signals are compensated.

6.7.1. Improved User Interface

The final interface allows to the user to select a communication port for the arduino card (visa resource), also allows to select a direction file to save the data results. Also, there is a progress bar to indicate the acquisition progress per crystal channel. There are two buttons, Start and Stop. The start button initiates the acquisition data and asks to the user by the fundamental frequency of the crystal in MHz. The resonance frequency of the crystal is obtained of its datasheet. The Figure 56 shows the final interface. The system was configured to work with crystals whose fundamental frequencies are between 1-30MHz.

There are two configuration modes: the standard and the advance. The standard mode selects automatically the bandwidth parameters and the step frequency of the sweep. The standard configuration is: bandwidth: 50000Hz and step frequency: 10Hz. Figure 57 shows the standard configuration.

Figure 56. final version of the user interface

The advance configuration allows to configure the bandwidth range and the step of the frequency to the sweep. To more accuracy, the delta of frequency can be until 1Hz. Finally, the interface asks to the user a cycles number for sweeping.

Figure 57. Variables to configure in final user interface

Figure 58. Panel to show results

Figure 58 shows the results interface. The signals of the conductance and susceptance are showing in the results. the series resonance frequency (F_s), Rho left (Γ_{left}), Rho right (Γ_{right}) and equivalent resistance (R_q) are obtained of the conductance graph. The frequency at minimum impedance with zero phase (F_r) is obtained of the susceptance graph.

6.7.2. Multichannel quartz crystal

A relays module was incorporated into the final version of the prototype. The purpose of this module is to switch the electronic acquisition circuit between different crystals connected to the module. The tests were done with four crystals. Arduino card was used to generate the control signals needed to select the crystal of interest. According to Figure 59, three double relays were necessary to switch four crystals.

The Figure 59 shows the relays schematic. There are three relays (U1, U2, U3) to switch between four crystals. The first one is connected to input signal through the toroid transformer and the reference signal (GND). Using the shown configuration for the relays, the input signal is addressed to a specific crystal.

Figure 59. Multichannel schematic in final prototype

In addition, the user can select more than one crystal for analyzing, including all the crystals. In these cases, the software switches the crystals beginning from the first one (crystal 1) to the last one (crystal 4). In other words, the software switches the crystals in descendent way.

The possibility of measure more than one crystal is a great advantage because each crystal can be used to characterize one specific liquid obtaining four independent measures. Moreover, each crystal can have different fundamental frequency with the same sample, In this case, the results would be a frequency spectrum; for instance: series resonance frequency (F_s) at 1MHz, 2 MHz, 5MHz and 10MHz.

6.7.3. Auto-Gain Adjustment System

In accordance with previous sections, to add liquid samples to the crystal surface generates an important damping in the crystal response. Therefore, the signal to noise ratio for the voltage, current and susceptance signals is a very important parameter for achieving a good resolution in the results. For improving this ratio, an auto-gain adjustment system has been created into the software. This “auto-gain function” into the Labview software permits in an automatic way to control the amplitude value for the input sinusoidal signal. For each cycle of measuring, the software evaluates the signals obtained with an optimum value of amplitude. In particular, for this prototype, the optimum value of amplitude is 4096 considering the resolution of the ADC of arduino DUE is 12 bits (2^{12}). Therefore, the peak amplitude of the signals is compared with a range close to the optimum value 4096. According to Figure 60, signals amplitude between 3900 to 4090 are considered with an optimum gain. Signals with a less amplitude value than 3900 require more gain and signals with more amplitude value than 4090 require less gain.

Figure 60. Representation of the current signal acquired in the three possible cases according to the auto-gain adjustment system.

Signals with amplitude more than 4096 appear saturated in the same way that in Figure 60 because the electronic acquisition system of the arduino does not permits values over than 3.3V analogous or 4096 digitals.

When the peak value of the signals is inside the “optimum range”, the auto-gain function maintains the same gain value for the amplification system. At hardware level, this gain value modifies the value of the digital resistor into an inverting amplifier. This amplifier is part of the amplifier system in the electronic card.

Using this auto gain module proposed, the damping related to viscous samples is compensated in the acquired signals. However, for very strong viscous samples, for instance, pure glycerol, the system does not achieve to compensate the losses. Therefore, all samples used in this work include aqueous dilutions.

6.7.4. Protocol of measure

The measurement protocol is as follows: to configure the frequency sweep using the user interface: (frequency range, sweep step and number of cycles). To calculate the series resonance frequency (F_0), the frequency at minimum impedance with zero phase (F_r), the half band half width ($\Gamma_{0\text{left}}$, $\Gamma_{0\text{right}}$) and the motional resistance (R_s) using the developed code in Labview for each cycle. These measures are taken ten times for the undamped crystal. The final values are the average of the ten measures. To put the sample to analyze on the electrode surface of the quartz crystal (50uL of volume), Can be in static mode or dynamic mode using a microfluidic cell. To adjust the frequency range in the user interface in order to obtain the admittance values of the quartz crystal in contact with the liquid sample considering the curves are shifted due to sample effect. For the results obtained in this work, the measures with sample (F_s , F_r' , Γ_s and R_s') are taken 100 times (aprox. 3 minutes) and the final values are the average of the measures. To obtain $\Delta F_s = F_s - F_0$, $\Delta F_r = F_r' - F_r$, $\Delta \Gamma = \Gamma_s - \Gamma_0$ and $\Delta R_s = R_s' / R_s$.

The density values were obtained using an analytical balance. The mass of each solution was found from a 1ml of its volume.

6.7.5. Protocol to clean crystals

Before each experiment, sensor crystal was placed in a Plasma cleaner (diener plasma surface model) for 10 min and immediately cleaned in acetone 96% and isopropyl alcohol 99.5% for 5 min then treated again with oxygen plasma for 1 min and rinsed with mili-q water.

6.7.6. Protocol to obtain the Biosensor Performance

The performance of the prototype was validated measuring mixtures of water and glycerol at different concentrations according to Table 7:

Concentration (%)	Density (mg/ml)	Viscosity (mPa·s)
40%	1106	3.25
30%	1054	2.25
20%	1048	1.69
10%	1023	1.35
5%	1010	1.13
2.5%	999	1.08
1.2%	1007	1.01
0.6%	1006	0.99
0.3%	998	0.98

Table 7. Glycerol dilutions data

The samples were prepared between glycerol and water milli-q according to the concentration needed. The total volume for each sample was 20ml because it is the minimum quantity to measure using the viscometer Fungilab with LCP spindle. Fungilab viscometer measures had been used as reference. The mixture was shaken a few minutes and characterized with the viscometer. The density value of the mixture was obtained using the microbalance for obtaining its mass. The volume was a known variable (1ml). The data are shown in Table 7.

In section 6.9 the viscosity obtained with the Fungilab viscometer is compared with the viscosity data found with QCR prototype. The sensitivity and limit of detection of the prototype are calculated in this section.

6.8. Results – Experimentation

Following the procedure described section 6.7.4, the principal variables of interest are obtained in experimental measures with an undamped crystal. Ten measures were obtained and the results of the mean and standard deviation are shown in Table 8.

Experiment	R_s (Ω)	F_s (Hz)	F_m (Hz)	F_r (Hz)	Γ_{left} (Hz)	Γ_{right} (Hz)	Γ_r (Hz)
Average	20	9970870	9970866	9970919	516	446	705
S.D	0.16	0.00	5.48	4.18	5.48	5.48	7.91

Table 8. Experimental results for undamped crystal (first use)

Experiment	R_s (Ω)	F_s (Hz)	F_m (Hz)	F_r (Hz)	Γ_{left} (Hz)	Γ_{right} (Hz)	Γ_r (Hz)
Average	41	9969909	9969909	9969925	860	673	976
S.D	2.46	16.57	19.94	32.26	32.25	17.29	33.38

Table 9. Experimental results for undamped crystal (months of use)

After calculating the variables of interest for the undamped crystal, a sample of water Mili-Q was deposited on the electrode of the crystal. Following the same procedure than the used for the unperturbed crystal; conductance and susceptance data for water samples were acquired. In this

case, the time for data reading is greater because the frequency interval increases to get all curves since the morphology of the curves changes, losing amplitude and increasing its bandwidth. The procedure was performed one hundred times. The average and standard deviation results are show in Table 10 and Table 11.

Experiment	R_s' (Ω)	F_s' (Hz)	F_m' (Hz)	F_r' (Hz)	Γ'_{left} (Hz)	Γ'_{right} (Hz)	Γ_r' (Hz)
Average	230	9968831	9968521	9969363	2763	2074	2646
S.D	0.39	5.56	18.05	44.71	33.03	14.62	49.70

Table 10. Experimental results for crystal in contact with water (first use)

Experiment	R_s' (Ω)	F_s' (Hz)	F_m' (Hz)	F_r' (Hz)	Γ'_{left} (Hz)	Γ'_{right} (Hz)	Γ_r' (Hz)
Average	252	9966924	9966819	9967638	3157	1986	2585
S.D	0.84	48.86	55.49	69.34	69.34	51.27	82.19

Table 11. Experimental results for crystal in contact with water (months of use)

	ΔR_s (Ω)	ΔF_s (Hz)	ΔF_m (Hz)	ΔF_r (Hz)	$\Delta \Gamma_{left}$ (Hz)	$\Delta \Gamma_{right}$ (Hz)	$\Delta \Gamma_r$ (Hz)
Experimental	11,8	-2038,8	-2345,0	-1556,4	2247,3	1627,6	1940,7

Table 12. “Deltas” of the experimental results (first use)

	ΔR_s (Ω)	ΔF_s (Hz)	ΔF_m (Hz)	ΔF_r (Hz)	$\Delta \Gamma_{left}$ (Hz)	$\Delta \Gamma_{right}$ (Hz)	$\Delta \Gamma_r$ (Hz)
Experimental	6.1	-2985	-3090	-2287	2297	1313	1609

Table 13. “Deltas” of the experimental results (months of use)

Figure 61. Crystal frequencies response in contact with water (First use)

Figure 62. Crystal frequencies response in contact with water (months of use)

Figure 63. Crystal Rhos (Γ) response in contact with water (First use)

Figure 64. Crystal Rho's (Γ) response in contact with water (months of use)

Using the results shown in Table 12 and Table 13 for calculating the viscosity of the sample (water):

$$\eta = 1.0 \text{ mPa}\cdot\text{s (using data in Table 12)}$$

$$\eta = 2.17 \text{ mPa}\cdot\text{s (using data in Table 13)}$$

The results suggest that a prolonged use of a same crystal, affects its performance. This effect may be also related to the cleaning protocol because if the crystal is not properly cleaning, the measures are altered due to wastes on the electrode. The coupling between the crystal and the electronic card is also fundamental in the correct acquisition of the variables.

The experimental results in air are compared with simulation results of the geometric model and BVD model for an undamped crystal. The results are shown in Table 14.

The experimental results in water are comparing with simulation results of the geometric model and BVD model for bare crystal. The results are shown in Table 15.

Indicator	Experimental (first use)	Experimental (months of use)	3D Model	BVD Model
Rs (Ω)	19 ± 0.07	91 ± 2.46	0.098	0.38
Fs (Hz)	9970870 ± 0.0	9969909 ± 16.57	9850700	9984543
Fm (Hz)	9970866 ± 5.78	9969909 ± 19.94	9850700	9984543
Fr (Hz)	9970919 ± 2.75	9969925 ± 32.26	9850700	9984543
Γ_{left} (Hz)	516 ± 5.48	860 ± 32.25	2.3	4
Γ_{right} (Hz)	446 ± 5.48	673 ± 17.29	2.3	4
Γ_r (Hz)	705 ± 7.91	976 ± 33.38	3	7
Quality Factor	10365	6503	2141456	1248067

Table 14. Comparison between experimental and simulation results for undamped crystal

Indicator	Experimental (first use)	Experimental (months of use)	3D Model	BVD Model
Rs' (Ω)	226 ± 0.28	252 ± 0.39	330	204,52
Fs' (Hz)	9968831 ± 5.56	9966924 ± 48.86	9848645	9982524
Fm' (Hz)	9968521 ± 18.05	9966819 ± 55.49	-	9982398
Fr' (Hz)	9969363 ± 44.71	9967638 ± 69.34	-	9982524
Γ'_{left} (Hz)	2763 ± 33.03	3157 ± 69.34	1835	2023
Γ'_{right} (Hz)	2074 ± 14.62	1986 ± 51.27	1835	2023
Γ_r' (Hz)	2646 ± 49.70	2585 ± 82.19	-	2023
Quality Factor	2061	1937	2683	2467

Table 15. Comparison between experimental and simulation results for crystal in contact with water

According to Table 14 and Table 15, the experimental results of Rs and Γ values suggest more losses than in simulations, this behavior is expected because the real prototype has external components than generate losses. For instance, in real life, the prototype is exposed to changes in temperature and external vibrations generating additional losses that in simulations were not into account. In addition, the comparison between experimental results with a new crystal “first use” and a crystal with a prolonged use suggests the prolonged use affect the crystal behavior, this affectation may be related with the cleaning procedure of the crystal and the coupling of the crystal and the electronic card. A small reduction in the quality factor is a consequence of these effect.

	ΔR_s (Ω)	ΔF_s (Hz)	ΔF_m (Hz)	ΔF_r (Hz)	$\Delta \Gamma_{\text{left}}$ (Hz)	$\Delta \Gamma_{\text{right}}$ (Hz)	$\Delta \Gamma_r$ (Hz)
First use	11,8	-2038.8	-2345.0	-1556.4	2247.3	1627.6	1940.7
Months of use	6.1	-2985	-3090	-2287	2297	1313	1609
3D Simulation	3367	-2055	-	-	1832.7	1832.7	-
BVD Simulation	538.2	-2019	2145	-2019	2019	2019	2019

Table 16. Comparison between experimental and simulation “deltas”

The results shown in Table 16 advise there are some variables that differ of the theory model. In particular, ΔR_s , ΔF_r and $\Delta \Gamma_{\text{right}}$ are the values with more difference. ΔR_s is related directly with the loses in the prototype, the results suggest there are additional loses in the prototype compared with simulations. It is possible because temperature changes, external vibrations, the cleaning protocol and the coupling between the crystal cell and the electronic card are variables that affect the behavior of the crystal. However, the principal variables (ΔF_s , $\Delta \Gamma_{\text{left}}$) included in equations for obtaining viscosity values save a close relation with the simulations results.

6.9. Sensibility and Limit of Detection of the Biosensor

According to section 6.7.6, the principal data of each concentration of glycerol-water mixtures were obtained and shown in Table 17.

The viscosity values have been obtained using the equation 45 with the frequency shift and density values got with analytical balance. The equation 45 is a simplified expression of the equation 43 supposing the sample has a Newtonian behavior. Glycerol-water mixtures can be considered Newtonian fluid.

Concentration (%)	ΔF_s (Hz)	$\Delta \Gamma_{\text{left}}$ (Hz)	Density (mg/ml)	Viscosity (mpa·s)
40%	-3660±49	3897±23	1106	3.168±0.08
30%	-2968±51	3172±12	1054	2.194±0.07
20%	-2617±86	2714±14	1048	1.664±0.05
10%	-2281±48	2277±33	1023	1.247±0.01
5%	-2064±32	2096±21	1010	1.053±0.03
2.5%	-2011±22	2042±28	999	1.010±0.03
1.2%	-2004±54	1953±33	1007	0.954±0.01
0.6%	-1920±49	1926±21	1006	0.902±0.01
0.3%	-1911±92	1943±18	998	0.914±0.06

Table 17. Viscosity results for glycerol aqueous dilutions

Figure 65. Δf_s and $\Delta \Gamma_{\text{left}}$ behavior for glycerol dilution samples.

In accordance with Kanazawa equation (equation 52), Figure 65 shows a lineal behavior for QCR prototype. In addition, the behavior between Δf_s and $\Delta \Gamma$ is the same practically, indicating Newtonian behavior for the sample.

Concentration (mg/ml)	QCR (mPa·s)	Viscometer (mPa·s)
504	3.168±0.08	3.25
378	2.194±0.07	2.25
252	1.664±0.05	1.69
126	1.247±0.01	1.35
63	1.053±0.03	1.13
31	1.010±0.03	1.08

15	0.954±0.01	1.01
7,9	0.902±0.01	0.99
3,9	0.914±0.06	0.98

Table 18. Comparison between QCR viscosity results and Fungilab viscometer

The viscosity values shown in Table 18 have been obtained using Kanazawa equation.

Considering the glycerol density is: 1.26 g/ml and knowing the volume of the mixtures, it is possible of expressing the concentration of glycerol in (mg/ml). The results are shown in the Table 18 and the Figure 66.

Figure 66. Response of the QCR prototype vs Fungilab reference

Table 18 and Figure 66. show the performance of the QCR at different samples. The viscosity values obtained by QCR prototype were compared with a conventional viscometer (Fungilab Smart series). According to Table 18, the QCR prototype has a behavior very similar in comparison with the Fungilab reference. This was a good result respect to the expectative. Both measurements (QCR and Fungilab) were done in simultaneous and at room temperature 20°C.

Figure 67. Biosensor response at different concentration sample

According to Figure 67, the sensibility of the QCR prototype is around 3.2 Hz per 1mg/ml of sample concentration change. The linear behavior of the QCR response was confirmed [46,106,129,130].

The limit of detection is calculated as three times the standard deviation of the sample without analyte (pure water in this case) evaluated in the sensitivity equation. In this case, the offset in the equation is not considered. Based on Table 10, the SD for pure water in contact with the crystal surface is around 5Hz. Therefore, the limit of detection for the QCR prototype is around 48mg/ml for glycerol dilutions. This results is in accordance with the Figure 67 where for values close to ~50 mg/ml the slope of the curve is inverted indicating more frequency shift for a sample with lower concentration (not reliable result).

6.10. Results – Relative Permittivity measure with QCR

The equation 88 and 89 in section 6.5.3 are solved with experimental data and BVD model values shown in section 6.3.2. Table 19 shows results of ten independent experiments with the capacitance C_s obtained for each one.

Experiment	R_s (ohm)	F_s (Hz)	Γ'_{left} (Hz)	C_s (F)
1	205,88	9983704	3111	8,48E-12
2	203,04	9983745	3036	7,61E-12
3	202,80	9983740	2887	7,13E-12
4	202,33	9983728	2840	7,76E-12
5	202,58	9983742	2957	7,22E-12
6	200,28	9983756	2842	9,18E-12
7	202,04	9983746	2814	9,18E-12

8	198,41	9983743	2853	8,55E-12
9	202,81	9983744	2882	7,44E-12
10	201,97	9983734	2853	7,44E-12
Average	202,20	9983738,16	2904,81	7,93E-12
SD	1,92	14,24	97,14	7,84E-13

Table 19. Results for relative permittivity measures for water

According to these results, the capacitance due to sample $C_s = 7.9 \pm 0.8$ pF. Using the equation proposed by Rodahl et al [88] ($C_s = 0.095\epsilon_r$ pF), is possible to relate the capacitance obtained with the relative dielectric constant for the water sample.

The dielectric constant obtained is $\epsilon_r = 83.47$. This value is very close to the official value for Milli-Q water which is 81. In this way is possible to find the relative permittivity of the sample using a QCR.

6.11. Discussion – Equivalent electric model proposed with C_s

In this section is proposed a modified electric model to the quartz crystal considering the capacitance C_s due to the liquid in contact with the electrode of the crystal.

Figure 68. Model proposed including the parallel capacitance related to the fluid sample

The model shown in Figure 68 is more accuracy in accordance with the experimental results. As it has been demonstrated in section 6.5.2, the sample in contact with crystal electrode generates a displacement of the frequency at minimum impedance with zero phase (F_r) respect to series resonant frequency (F_s). According to section 6.5.3, this displacement can be related with a stray capacitance (C_s) in parallel to static capacitance C_0 .

According to results, the capacitance C_s can be a consequence of the relative permittivity difference between the environment and the sample. In tests case: air and water. For the undamped crystal, the stray capacitance C_s does not exist because the relative air permittivity is less than the quartz crystal. But the water has many more permittivity that the crystal, around 22 times.

This phenomenon modifies the static capacitance C_0 increasing its value. For this reason, in Figure 68 is proposed the stray capacitance in parallel to the static capacitance, because the C_0^* obtained experimentally is greater than C_0 , for undamped crystal. However, this potential application is limited to samples with high permittivity in comparison with air and quartz.

7. Chapter III – The Proposed Biomedical Application: Potential diagnosis of arthritic disease

According to CHAPTER 1 - Fundamentals and QCR Theory, Quartz Crystal Resonators (QCR) are devices capable of characterizing samples according to their viscosity. In this chapter, a biomedical application proposed using this capability for the diagnosis of arthritic disease through the analysis of the synovial fluid. How probable advantages for the user are: real time diagnosis, low cost technology, sensor easy to use, user interface can operate in any computer. In addition, this technique can help to design treatment protocols for patients because the sensor permits compare the viscosity values over time. This comparison helps to see the evolution of the patient related to the treatment without complex laboratory techniques. How advantages for patients: the sensor does not require more than some tens of microliters of sample, that means, least discomfort in the extraction of the sample. In case of high risk patients (sport people or older people), this sensor can provide an easy technique to know the status of their joints of regular manner.

7.1. Introduction

According to the Mayo clinic[131], arthritis disease is inflammation of one or more of the joints. The main symptoms of arthritis are joint pain and stiffness, which typically worsening with age. The most common types of arthritis are osteoarthritis and rheumatoid arthritis. However, arthritis disease is very common concept but is not well understood. In fact, “arthritis” is not a single disease; it is an informal way of referring to joint pain or joint inflammation.

There are many different types of arthritis and related conditions. People of all ages, sexes and races can have arthritis, and it is the leading cause of disability in America. More than 50 million adults and 300,000 children have some type of arthritis.[7,132] Common arthritis joint symptoms include swelling, pain, stiffness and decreased range of motion. Symptoms may come and go. They can be mild, moderate or severe. They may stay about the same for years, but may progress or get worse over time. Severe arthritis can result in chronic pain, inability to do daily activities and make it difficult to walk or climb stairs. Arthritis can cause permanent joint changes.[131,132] These changes may be visible, such as knobby finger joints, but often the damage can only be seen on X-ray. Some types of arthritis can also affect the heart, eyes, lungs, kidneys and skin as well as the joints.

Arthritis pain is the second cause of critical disability (behind respiratory illness) and is the number one cause of chronic disability in the general population. More than 45 million adults have been diagnosed with arthritis in North America.[7] Of working-age adults (aged 18 to 64 years) with doctor-diagnosed arthritis, 31% have arthritis-attributable work limitations. Because these are prime working years, musculoskeletal conditions cause significant loss of work productivity. Overall, a quarter (25%) of social security disability payments are related to rheumatologic disorders making it the leading cause of social security disability payments.[7]

The most common types of arthritis are: Degenerative Arthritis or osteoarthritis and Inflammatory Arthritis or rheumatoid arthritis. Osteoarthritis is a slowly progressive, non-inflammatory musculoskeletal disorder that typically affects the joints of the hand, spine, and weight-bearing

joints of the lower extremity.[7] The cartilage wears away; in consequence, the bone rubs against bone, causing pain, swelling and stiffness. Over time, joints can lose strength and pain may become chronic. Risk factors include excess weight, family history, age or a previous injury.[132]

In rheumatoid arthritis (RA), the immune system can go twisted, mistakenly attacking the joints with uncontrolled inflammation, potentially causing joint erosion and may damage internal organs, eyes and other parts of the body.[132] According with [7], RA is a chronic, systemic, inflammatory disorder of unknown etiology that is characterized by its pattern of di-arthrodial joint involvement. Its primary site of pathology is the synovium of the joints. The synovial tissues become inflamed and proliferate, forming pannus that invades bone, cartilage, and ligaments and leads to damage and deformities. Researchers believe that a combination of genetics and environmental factors can trigger autoimmunity. Smoking is an example of an environmental risk factor that can trigger rheumatoid arthritis in people with certain genes.[132]

For diagnosis of Osteoarthritis, the typical tests are: Erythrocyte sedimentation rate (ESR), Rheumatoid factor (RF), antinuclear antibodies (ANAs), radiographic images. In the case of RA, the principal laboratory tests are: Complete Blood Count (CBC), Erythrocyte sedimentation rate (ESR), Rheumatoid factor (RF), Anti-CCP, Antinuclear antibodies (ANA), ANCA, Complement (C3, C4, CH50).[7] However, all these tests are specific laboratory tests usually expensive, laboratory people trained are needed and these tests are no common to do until the patient refers pain and the disease is advanced. Nowadays in many countries including Colombia, the main technique to diagnose arthritic diseases is the radiographic image. This technique is less expensive than others commented but it has the same inconvenient, the diagnostic is too late because in a radiographic image the arthritic disease is evident only when the problem is in an advance status. On the other hand, the arthritic diseases are related with changes in the synovial fluid.[6–9,11]. But currently, the synovial fluid is not an efficient source of information because its analysis is commonly expensive. Therefore, in this work, it is proposed a synovial fluid analysis with the aim of contributing with information about the status of patient joints.

The synovial fluid is produced by the synovium membrane inside the articular cartilage. It can be considered like a dialysate of plasma with high content of hyaluronic acid. [4–6] The main functions of synovial fluid are the lubrication of the joints and the absorption of mechanical load produced when walking or running (knees) or holding up weight (cubit and shoulder). [4–6,8] The viscosity of the synovial fluid depends on the concentration of the hyaluronic acid (section 4.6). There are studies that relate the low viscosity of the synovial fluid (low concentration of hyaluronic acid) with arthrocentesis, osteoarthritis and inflammatory arthropathies [4–6]. Also, the quantity of the synovial fluid in a joint is not bigger than 3.5ml (knee)[8].

In joint diseases, the hyaluronic acid quantity decreases in the synovial fluid generating a bad lubrication function and a less damping of the mechanical load against the physical stress.[68] Due to this affectation, the viscosity analysis of the synovial fluid can contribute with information for the diagnostic of some arthritic pathologies.[13]

Nowadays, in cases when the synovial fluid is analyzed, this analysis is done through macroscopic techniques (color, transparency and viscosity), microscopic analysis (count cells, leucocytes, etc.) and special analysis (glucose, crystals, etc)[5,6] According to Table 20, the viscosity is a differential factor at the classification of the samples between normal, inflammatory, septic, or hemorrhagic [5,12,133]. However, just in qualitative way.

	Normal	Inflammatory	Septic	Hemorrhagic
Appearance	Transparent	Turbid	Turbid	Bloody
Color	Straw-colored	Yellow-cloudy	Opaque	Red-opaque
Viscosity	High	Low	Low	Variable

Table 20. Macroscopic classification of synovial samples

The test of the viscosity involves of taking the sample extending it on a surface and measuring the length before the thread breakage. If the final length is greater than 3cm, the sample is healthy, if else; the sample indicates some problem and more tests are needed.[8]

This current method does not permit to obtain a value of the viscosity and relate it with any arthritic disease. Therefore, in this work, it is proposed of obtaining values of viscosity for samples elaborated using hyaluronic acid (HA) in aqueous dilutions according to section 7.2.

7.2. Elaboration of the Samples

About the samples used, according to Sterling W,[7] synovial means “like egg white,” which describes the normal viscosity of synovial fluid. [7] Therefore, egg white and milli-q water mixtures at different concentrations were used to elaborate some samples. In addition, due to the difficulty for obtaining real samples of synovial fluid of patients with arthritic disease in preliminary tests. Additional samples were done with HA mixtures and milli-q water with the aim of emulating the synovial fluid behavior.

The albumin egg was obtained from fresh eggs. Egg white dilutions at different concentrations (20%, 40%, 60%, 80% 100%) have been used in order to emulate the behavior of synovial fluid when it has high viscosity (healthy) or it has low viscosity (inflammatory, hemorrhagic or septic). In similar way, samples of hyaluronic acid aqueous dilutions have been used too. The hyaluronic acid was supplied by sigma Aldrich, Mw=1,5MDa. The concentration of the hyaluronic acid in the healthy synovial fluid is around 3.5mg/ml.[9,134,135] In traumatic arthritis and Osteoarthritis, the hyaluronic acid concentration decreases around to 1.3mg/ml and for rheumatoid arthritis the concentration is lower, just 0.84mg/ml.[9]. Considering these data, the hyaluronic acid samples have been done with the same concentrations in order to simulate the specific diseases. One additional sample to 7mg/ml was done with the purpose of obtaining an extra point in the analysis of results. Just 50uL of volume of the sample is needed to deposit on the electrode surface to perform the analysis.

The hyaluronic acid samples were diluted in cold water in order to avoid creation of clots and facility the dilution. These samples need 8 hours inside the refrigerator to 5 centigrade to be ready.

Due to albumin egg, HA dilutions and the synovial fluid have a non-Newtonian behavior (viscoelastic and pseudoplastic), [8,68] was necessary to use equations more general than Kanazawa equation. In particular, the viscoelastic model analyzed in Non-Newtonian Fluids section.

7.3. Results – Egg white samples

According to the measurement protocol (section 6.7.4) samples of egg white have been analyzed. Table 21 shows the QCR response values at different dilutions of egg white. According to the literature, the egg white is a typical non-Newtonian fluid with low molecular weight (around

42.7kDa)[136] and pseudoplastic behavior (decrease the viscosity to increase the shear rate) [137].

Concentration %	ΔF_s (Hz)	$\Delta \Gamma_{left}$ (Hz)	Density (mg/ml)
100	-3175±79	3005±69	1043
80	-2980±68	2997±71	1034
60	-2878±82	2567±61	1026
40	-2771±72	2390±66	1017
20	-2579±77	2209±68	1036

Table 21. Data for white egg samples (average±SD)

On the other hand, to obtain the correction factor χ , the following data (Table 22) were used in conjunction with equations shown in section 4.5 and 4.5.1:

Constant	value
Gas constant -R (kg m ² s ⁻² K ⁻¹ mol ⁻¹)	8.314
Molecular Weight (Da)	4.27 x 10 ⁴
Temperature (K)	288
Solvent viscosity mPa·s	1.00
ν (For Rouse Model)	2.0
Riemann zeta function (ν)	1.645
Quartz Density (mg/ml)	2651
Shear modulus in quartz (Pa)	2.9340 x 10 ¹⁰

Table 22. Constants used for obtaining correction factor χ .

Density	Concentration	$\Sigma n'$	$\Sigma n''$	η'/η_0	η''/η_0	$\tan(\delta)$	Factor χ
1043	100,0	1,61	1,06	0,981	0,100	9,8	0,941
1034	80,0	1,62	1,07	0,988	0,074	13,4	0,957
1026	60,0	1,63	1,08	0,993	0,041	24,1	0,976
1017	40,0	1,64	1,08	0,995	0,025	40,4	0,985
1036	20,0	1,64	1,08	0,995	0,013	75,9	0,991

Table 23. Principal variables for obtaining the correction factor χ .

Following the process explained in section 4.5 and 4.5.1, the results shown in Table 22 are obtained. Based on these results, the egg white dilutions have a behavior Newtonian practically. The low (Mw) is an indication of the same result. In addition, considering that the loss tangent $\tan(\delta)$ quantifies the balance between energy loss and storage, a value for $\tan(\delta)$ greater than unity indicates more "liquid" properties and, according to the results, $\tan(\delta)$ values indicate almost total liquid behavior (viscous behavior). Therefore, the correction factor χ is the unity practically.

The QCR data shown in Table 21 are used to obtain the apparent viscosity using the equation (48) without correction factor χ and with equation (59) after Rouse model correction in order to compare the results. These results are shown in Table 24.

Concentration %	Viscometer (mPa·s)	QCR without Rouse Model (mPa·s)	QCR with Rouse Model (mPa·s)
100	8.28	6.34±0.06	7.08±0.06
80	7.12	5.51±0.04	5.84±0.08
60	6.17	5.00±0.05	4.91±0.05
40	5.49	4.23±0.06	4.35±0.07
20	4.54	3.65±0.09	3.67±0.09

Table 24. Results for egg white dilutions without and with Rouse Model factor correction

In Figure 69 is noticeable the effect to apply the Rouse model. Effectively, the Rouse model correction permits to obtain values closer to the reference viscometer data.

Figure 69. Apparent viscosity obtained applying the Rouse model for white egg samples

According to Figure 69, the viscosity behavior before and after correction factor χ is very similar. That means the white egg fluid has a behavior close to the Newtonian model. A possible explanation for this phenomenon is the quality of the egg. According to [137], the thin egg white is a very low viscous liquid with a viscosity between 5 and 10 mPa·s and its flow behavior is very similar to that of an ideal Newtonian liquid. How the percentage of albumin decrease in the solution, the factor χ is closer to the unity. In other words, the more diluted sample is more similar to Newtonian fluid, in this case: the water (the solvent).

7.4. Results – Hyaluronic acid samples

According to the methods exposed, the following data were obtained for the hyaluronic acid mixtures:

Constant	value
Gas constant -R (kg m ² s ⁻² K ⁻¹ mol ⁻¹)	8.314
Molecular Weight (Da)	1.5 x 10 ⁶
Temperature (K)	288
Solvent viscosity mPa·s	1.00
v (For Rouse Model)	2.0
Riemann zeta function (v)	1.645
Quartz Density (mg/ml)	2651
Shear modulus in quartz (Pa)	2.9340 x 10 ¹⁰

Table 25. Constants used for obtaining correction factor χ for HA dilutions.

Table 25 shows the principal variables for obtaining the correction factor χ for HA dilutions. In comparison with egg white dilutions, the main difference is the change in the Molecular weight. In case of HA dilutions, the Mw is increased 2 magnitude orders.

Concentration H.A (mg/ml)	ΔF_s (Hz)	$\Delta \Gamma_{left}$ (Hz)	Density (mg/ml)	viscosity (η_0) (mPa·s)
7,00	-2691.4±86	3615±15	1032.23	12745
3,50	-2617.4±48	3563±13	1017.89	7349.7
1,29	-2563.6±60	3463±15	1014.32	355.01
0,85	-2556.6±84	3341±9	1019.53	161.33

Table 26. Hyaluronic Acid (HA) results and Zero-shear viscosity (Average $\pm$ SD)

Table 26 shows the initial results obtained using the QCR prototype. The density is obtained using the analytic balance weighting 1ml of each sample. The zero-shear viscosity (η_0) has been obtained using the Fungilab viscometer at very low shear rate (5mHz). ΔF_s and $\Delta \Gamma_{left}$ corresponds to the average of 20 independent measures for each sample. At the same time, each measure is the average of 100 cycles of sensing. In HA dilutions case, the pseudoplastic behavior is more evident than egg white. Moreover, the ΔF_s and $\Delta \Gamma_{left}$ values are the same for water deposition.

Concentration H.A (mg/ml)	Density (mg/ml)	η'/η_0	η''/η_0	$\tan(\delta)$	Factor χ
7.00	1032	0.003	0.003	0.990	0.04
3.50	1017	0.007	0.007	0.999	0.05
1.29	1010	0.010	0.010	1.002	0.06
0.85	997	0.010	0.010	1.003	0.06

Table 27. Principal variables for obtaining the correction factor χ for HA dilutions.

Table 27 shows the results of factor χ for each dilution of HA. Analyzing these results, loss tangent values are close to the unity. These results indicate both liquid and solid properties at the same proportion (viscoelastic behavior). Due to the loss tangent is very close to the unity, the elastic properties in the samples are very important too. In other words, the viscoelastic behavior is evident for HA dilutions. In consequence, the factor χ results low in comparison to the unity.

However, in accordance with the results, if the sample is more diluted (any arthritic disease) the factor χ is closer than the unity indicating more viscous than elastic behavior.

Concentration H.A (mg/ml)	Δf_s (Hz)	Δf_s Adjusted (Hz)
7.00	-2691.4 $\pm$ 86	-74229.6
3.50	-2617.4 $\pm$ 48	-48642.9
1.29	-2563.6 $\pm$ 60	-40551.5
0.85	-2556.6 $\pm$ 84	-39709.5

Table 28. Δf_s measured and adjusted with correction factor χ

Table 28 shows the Δf_s obtained with the QCR prototype and Δf_s adjusted using the correction factor χ . These new values correspond to the frequencies shift for each sample if the samples behavior were only viscous. In other words, if the samples had Newtonian behavior.

Concentration %	Viscometer (mPa·s)	QCR without Rouse Model (mPa·s)	QCR with Rouse Model (mPa·s)
7.00	2311.6	2.42 $\pm$ 0.26	1312.4
3.50	417.9	2.32 $\pm$ 0.18	571.9
1.29	126.7	2.21 $\pm$ 0.14	400.2
0.85	94.2	2.13 $\pm$ 0.09	388.8

Table 29. Results for HA dilutions without and with Rouse Model factor correction

Table 29 shows the apparent viscosity obtained using the equation 48 with the frequency shift Δf and bandwidth shift $\Delta \Gamma$ measured (Table 26). In accordance with Table 29, viscosity values after adjusted using the correction factor, they are more comparable with viscometer measures. These viscometer measures were obtained with Fungilab Smart at 12rpm.

Figure 70. Apparent Viscosity obtained by Rouse model vs viscometer measures.

Figure 70 shows the comparison between the apparent viscosity obtained by Rouse model using the QCR measures and the viscometer measures.

7.5. Discussion

The QCR prototype proposed has a good performance with egg with dilutions, the results illustrate the capability of the QCR sensor to measure small changes in the composition of egg white aqueous dilutions adjusting the results with correction factor χ close to the unity. These results shown the egg white dilutions had almost Newtonian behavior.

According to the results using hyaluronic acid dilutions, the samples shown a complex rheological behavior, these solutions are cataloged into the pseudoplastic fluids. Initial results show that it is not possible to compare to high shear rate (QCR measures) and low shear rate (viscometer measures). The QCR measures of hyaluronic acids dilutions require an additional factor χ for obtaining results comparable with viscometer data. Rouse model (section 4.5) is a good alternative for obtaining the additional factor χ and adjusting the experimental measures.

Applying the Rouse model to the experimental results, new apparent viscosities were obtained for each sample. The new apparent viscosities related just to viscous component of hyaluronic acid sample have comparable behavior than viscometer measures (Figure 70). However, for the more concentrated sample (7mg/ml) the viscometer Fungilab generated a higher value than the QCR value adjusted. It is possible that the measure had been obtained with a very low shear rate.

In accordance with the theory proposed by Nwankwo and coworkers[66] the apparent viscosities obtained after the use of correction factor χ were calculated using the kanazawa equation considering the new frequency shifts correspond to an equivalent fluid just with viscous component.

The obtained results confirm the relationship between hyaluronic acid concentration and the viscosity of the fluid. Considering the concentration samples used in this work are very similar to reported for arthritic diseases[9,134] and the results shown, the QCR biosensor would be capable to identify synovial fluid samples (healthy or with any arthritic affectation).

7.5.1. Additional Considerations

In the experiments done, the correction factor calculated was applied to each QCR measure using the HA concentration as variable of relation. In other words, knowing the HA concentration of the sample was relative simple to apply the correction in the data. However, for future real samples, the HA concentration will be unknown. For these cases, the variables for relating the correction factor and the QCR shift frequency will be the equivalent resistance R_q and Γ_{left} .

Concentration (mg/ml)	R_q ohms	Δf_s	Δf_m	Δf_r	Γ_{left}	Γ_{right}	Γ_B
7	164.2±3.2	-2691.4±86	-2834.4±81	-2490.6±87	3615±15	1628.7±26	2219.5±43
3,5	152.3±2.1	-2617.4±48	-2751.6±48	-2462.0±39	3563±13	1558.8±22	2078.8±32
1,75	147.7±2.7	-2563.6±60	-2673.8±69	-2369.1±42	3463±15	1459.7±26	1993.0±29
0,85	141.4±2.2	-2556.6±84	-2663.0±55	-2378.4±35	3341±9	1443.7±34	1987.3±26

Table 30. Additional results for HA dilutions using QCR prototype

According to Table 30, R_q and Γ_{left} are variables which can be used for relating the correction factor and the QCR frequency shift because are directly proportional to the concentration values. The other variables are not useful because they have a very high SD.

8. CONCLUSIONS

The prototype developed has a performance that permit obtain good results in accordance with the theory models. The technique used based on Nakamoto[42] classified into the Lock-In Techniques permitted to obtain all variables of interest related to the crystal behavior. In general terms, the experimental results have good correlation with simulation results. Moreover, the time needed for obtaining the results data is only a couple minutes. This is a very good advantage of the device because permits obtain the results in real time.

The quantity needed to obtain the results was some tens of microliters. For biomedical applications, as the proposal in this work, this is a very clear advantage because the available quantities of biological samples usually are very small.

The comparison between a quartz crystal used for the first time and a quartz crystal with months of use demonstrates the performance of the crystal is affected with the use, especially in the SD values. This result may be related with the cleaning protocol. However, the recommendation is to use new crystals or crystal with little use.

The experimental results obtained using the developed device permitted to find an additional capacitive effect (stray capacitive) related to the permittivity of the sample. The effect was validated with water as the reference sample and the results were compared using the equation proposed by Rodahl and coworkers [88]. In relation to this effect, the permittivity could be measured with a minimum of error. However, this interesting application can be applied just with high permittivity value samples.

The device has been tested with glycerol and water milli-q mixtures at different concentrations with the aim of obtaining its sensibility and limit of detection considering the mixtures samples as a reference. The results shown the linearity of the response of the QCR prototype in accordance with the theory. The limit of detection was 48mg/ml for glycerol dilutions. The sensibility obtained was 4Hz per 1mg/ml. Both sensitivity and the limit of detection can be improved controlling some external variables, in particular the external vibrations presents close to the prototype.

The HA dilutions results demonstrate the QCR prototype can be used to analyze pseudoplastic samples using the correction factor χ to adjust the responses. Although the responses obtained are not the same than the values obtained with the Fungilab viscometer. Both values can be compared and each viscosity value for each concentration is sufficiently different from each other for permitting the characterization of the sample. In other words, and in accordance with the Figure 70, the viscosity values adjusted with the factor χ differ significantly to classify each sample between “healthy” or “abnormal”.

9. FUTURE WORK

As future work, there are the following perspectives:

- Design and validate a wireless system of communication, bluetooth probably, for sending and receiving data between the prototype and the computer. This advantage will permit of putting the prototype system in a controlled space (free of external vibrations especially) and the person who are measuring, in another environment. This development will be done with the aim of improving the limit of detection and the sensitivity of the sensor. In addition, this advantage will also permit of monitoring special events that require specific environments. For instance, cellular growing.
- Validate new crystals with different resonant frequency with the aim of comparing responses and searching additional information.
- In the San Jose hospital in Bogotá, Colombia, there are an investigation group of rheumatologists interested in contributing with real synovial fluid samples of patients with osteoarthritis and rheumatic arthritis with the aim of validating the performance of the prototype in a real scenario. Based on these tests, a new prototype will be done in order to minimize the electronic card. This advantage will permit to rheumatologists to count with a portable sensor as an additional tool to diagnosis arthritic diseases based on synovial fluid analysis. Probably, the system should be patented in this scenario.
- When the real samples are available, additional variables as R_q and Γ_{left} will be consider to relate the correction factor with the QCR response with the aim of obtaining an equivalent viscosity related just with viscous properties.

REFERENCES

1. Sauerbrey, G. Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung. *Zeitschrift für Phys.* **1959**, *155*, 206–222.
2. Keiji Kanazawa, K.; Gordon, J. G. The oscillation frequency of a quartz resonator in contact with liquid. *Anal. Chim. Acta* **1985**, *175*, 99–105.
3. Johannsmann, D. *The Quartz Crystal Microbalance in Soft Matter Research*; Soft and Biological Matter; 1st ed.; Springer: Gewerbestrasse, Switzerland: Cham, 2015; Vol. 53.
4. Pascual, E.; Jovani, V. Synovial fluid analysis. *Best Pract. Res. Rheumatol.* **2005**, *19*, 371–386.
5. Brannan, S. R.; Jerrard, D. A. Synovial fluid analysis. *J. Emerg. Med.* **2006**, *30*, 331–339.
6. Denton, J. Synovial fluid analysis in the diagnosis of joint disease. *Diagnostic Histopathol.* **2012**, *18*, 159–168.
7. Sterling, W. *Rheumatology Secrets*; Third Edit.; Elsevier: Philadelphia, PA, USA, 2015.
8. Araceli, M.-C.; Núñez, C.; Cabiedes, J. Synovial fluid analysis. *Reumatol. Clínica* **2010**, *6*, 316–321.
9. Stafford, C.; Niedermeier, W.; Holley, H.; Pigman, W. Studies on the concentration and intrinsic viscosity of hyaluronic acid in synovial fluids of patients with rheumatic diseases. *Ann. Rheum. Dis.* **1964**, *23*, 152–7.
10. Barnett, C. H. MEASUREMENT AND INTERPRETATION OF SYNOVIAL FLUID VISCOSITIES. *Ann. Rheum. Dis.* **1958**, 229–234.
11. Gatter, R. A.; Andrews, R. P.; Cooley, D. A.; Fiechtner, J. J.; Minna, D. A.; Phelps, P.; Wasner, C. American college of rheumatology guidelines for performing office synovial fluid. *J. Clin. Rheumatol.* **1995**, *1*, 194–196.
12. Gallo Vallejo, F. J.; Giner Ruiz, V. [Diagnosis: synovial fluid analysis]. *Aten. Primaria* **2014**, *46 Suppl 1*, 29–31.
13. Swan, A. The value of synovial fluid assays in the diagnosis of joint disease: a literature survey. *Ann. Rheum. Dis.* **2002**, *61*, 493–498.
14. Oberfrank, S.; Drechsel, H.; Sinn, S.; Northoff, H.; Gehring, F. Utilisation of Quartz Crystal Microbalance Sensors with Dissipation (QCM-D) for a Clauss Fibrinogen Assay in Comparison with Common Coagulation Reference Methods. *Sensors* **2016**, *16*, 282.
15. Sinn, S.; Muller, L.; Drechsel, H.; Wandel, M.; Northoff, H.; Ziemer, G.; Wendel, H. P.; Gehring, F. K. Platelet aggregation monitoring with a newly developed quartz crystal microbalance system as an alternative to optical platelet aggregometry. *Analyst* **2010**, *135*, 2930–2938.
16. Stefan Sinn, M. H. Blood Coagulation Thromboplastine Time Measurements on a Nanoparticle Coated Quartz Crystal Microbalance Biosensor in Excellent Agreement with Standard Clinical Methods. *J. Biosens. Bioelectron.* **2013**, *4*, 4–9.
17. Hussain, M.; Northoff, H.; Gehring, F. K. QCM-D providing new horizon in the domain of sensitivity range and information for haemostasis of human plasma. *Biosens. Bioelectron.* **2015**,

18. Yao, C.; Qu, L.; Fu, W. Detection of fibrinogen and coagulation factor VIII in plasma by a quartz crystal microbalance biosensor. *Sensors (Basel)*. **2013**, *13*, 6946–6956.
19. Mustafa, M. K.; Nabok, A.; Parkinson, D.; Tothill, I. E.; Salam, F.; Tsargorodskaya, A. Detection of ??-amyloid peptide (1-16) and amyloid precursor protein (APP770) using spectroscopic ellipsometry and QCM techniques: A step forward towards Alzheimers disease diagnostics. *Biosens. Bioelectron.* **2010**, *26*, 1332–1336.
20. Wang, Y.; Moss, M. A. Effect of Resveratrol and Derivatives on Interactions between Alzheimer's Disease Associated A β Protein Oligomers and Lipid Membranes: A Quartz Crystal Microbalance Analysis. *Biophys. J.* **2016**, *110*, 256a-.
21. Uludağ, Y.; Tothill, I. E. Development of a sensitive detection method of cancer biomarkers in human serum (75%) using a quartz crystal microbalance sensor and nanoparticles amplification system. *Talanta* **2010**, *82*, 277–282.
22. Nowacki, L.; Follet, J.; Vayssade, M.; Vigneron, P.; Rotellini, L.; Cambay, F.; Egles, C.; Rossi, C. Real-time QCM-D monitoring of cancer cell death early events in a dynamic context. *Biosens. Bioelectron.* **2014**, *64*, 469–476.
23. Bianco, M.; Aloisi, A.; Arima, V.; Capello, M.; Ferri-Borgogno, S.; Novelli, F.; Leporatti, S.; Rinaldi, R. Quartz crystal microbalance with dissipation (QCM-D) as tool to exploit antigen-antibody interactions in pancreatic ductal adenocarcinoma detection. *Biosens. Bioelectron.* **2013**, *42*, 646–652.
24. Kim, Y. J.; Rahman, M. M.; Lee, J. J. Ultrasensitive and label-free detection of annexin A3 based on quartz crystal microbalance. *Sensors Actuators, B Chem.* **2013**, *177*, 172–177.
25. Salam, F.; Uludag, Y.; Tothill, I. E. Real-time and sensitive detection of Salmonella Typhimurium using an automated quartz crystal microbalance (QCM) instrument with nanoparticles amplification. *Talanta* **2013**, *115*, 761–767.
26. Hiatt, L. A.; Cliffel, D. E. Real-time Recognition of Mycobacterium tuberculosis and Lipoarabinomannan using the Quartz Crystal Microbalance. *Sens. Actuators. B. Chem.* **2012**, *174*, 245–252.
27. Wang, R.; Li, Y. Hydrogel based QCM aptasensor for detection of avian influenza virus. *Biosens. Bioelectron.* **2013**, *42*, 148–155.
28. Li, D.; Wang, J.; Wang, R.; Li, Y.; Abi-Ghanem, D.; Berghman, L.; Hargis, B.; Lu, H. A nanobeads amplified QCM immunosensor for the detection of avian influenza virus H5N1. *Biosens. Bioelectron.* **2011**, *26*, 4146–4154.
29. Xie, J.; Wang, H.; Lin, Y.; Zhou, Y.; Wu, Y. Highly sensitive humidity sensor based on quartz crystal microbalance coated with ZnO colloid spheres. *Sensors Actuators, B Chem.* **2013**, *177*, 1083–1088.
30. Korposh, S.; Selyanchyn, R.; Lee, S. W. Nano-assembled thin film gas sensors. IV. Mass-sensitive monitoring of humidity using quartz crystal microbalance (QCM) electrodes. *Sensors Actuators, B Chem.* **2010**, *147*, 599–606.
31. Mumyakhmaz, B.; Özmen, A.; Ebeoğlu, M. A.; Taşaltin, C.; Gürol, I. A study on the development of a compensation method for humidity effect in QCM sensor responses. *Sensors Actuators, B Chem.* **2010**, *147*, 277–282.
32. Yao, Y.; Chen, X.; Guo, H.; Wu, Z. Graphene oxide thin film coated quartz crystal

- microbalance for humidity detection. *Appl. Surf. Sci.* **2011**, *257*, 7778–7782.
33. Harbeck, M.; Erbahar, D. D.; Gürol, I.; Musluoğlu, E.; Ahsen, V.; Öztürk, Z. Z. Phthalocyanines as sensitive coatings for QCM sensors operating in liquids for the detection of organic compounds. *Sensors Actuators B Chem.* **2010**, *150*, 346–354.
 34. Harbeck, M.; Erbahar, D. D.; Gürol, I.; Musluolu, E.; Ahsen, V.; Öztürk, Z. Z. Phthalocyanines as sensitive coatings for QCM sensors: Comparison of gas and liquid sensing properties. *Sensors Actuators, B Chem.* **2011**, *155*, 298–303.
 35. Erbahar, D. D.; Gürol, I.; Gümüş, G.; Musluoglu, E.; Öztürk, Z. Z.; Ahsen, V.; Harbeck, M. Pesticide sensing in water with phthalocyanine based QCM sensors. *Sensors Actuators, B Chem.* **2012**, *173*, 562–568.
 36. Dewar, R.; Joyce, M. The quartz crystal microbalance as a microviscometer for improved rehabilitation therapy of dysphagic patients. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* **2005**, *3*, 2511–5.
 37. Carvajal Ahumada, L. A.; Peña Pérez, N.; Herrera Sandoval, O. L.; del Pozo Guerrero, F.; Serrano Olmedo, J. J. A new way to find dielectric properties of liquid sample using the quartz crystal resonator (QCR). *Sensors Actuators A Phys.* **2016**, *239*, 153–160.
 38. Höök, F.; Kasemo, B.; Nylander, T.; Fant, C.; Sott, K.; Elwing, H. Variations in coupled water, viscoelastic properties, and film thickness of a Mefp-1 protein film during adsorption and cross-linking: a quartz crystal microbalance with dissipation monitoring, ellipsometry, and surface plasmon resonance study. *Anal. Chem.* **2001**, *73*, 5796–804.
 39. Carvajal Ahumada, L. A.; González, M. X. R.; Sandoval, O. L. H.; Olmedo, J. J. S. Evaluation of Hyaluronic Acid Dilutions at Different Concentrations Using a Quartz Crystal Resonator (QCR) for the Potential Diagnosis of Arthritic Diseases. *Sensors (Basel)*. **2016**, *16*.
 40. Hu, W.; Chen, S.; Liu, L.; Ding, B.; Wang, H. Formaldehyde sensors based on nanofibrous polyethyleneimine/bacterial cellulose membranes coated quartz crystal microbalance. *Sensors Actuators, B Chem.* **2011**, *157*, 554–559.
 41. Andreeva, N.; Ishizaki, T.; Baroch, P.; Saito, N. High sensitive detection of volatile organic compounds using superhydrophobic quartz crystal microbalance. *Sensors Actuators, B Chem.* **2012**, *164*, 15–21.
 42. Nakamoto, T.; Kobayashi, T. Development of circuit for measuring both Q variation and resonant frequency shift of quartz crystal microbalance. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **1994**, *41*, 806–811.
 43. World Health Organization OMS | «Envejecer bien», una prioridad mundial <http://www.who.int/mediacentre/news/releases/2014/lancet-ageing-series/es/>.
 44. Sociedad Española de Reumatología ¿Qué es la Artritis Reumatoide? Enfermedades Reumáticas http://www.ser.es/pacientes/enfermedades_reumaticas/artritis_reumatoide.php.
 45. Alcaide, F.; Esteban, J.; González, J.; Palacios, J. J. ... *Procedimientos en Microbiología Clínica* 2005,.
 46. Cassiède, M.; Daridon, J.-L.; Paillol, J. H.; Pauly, J. Characterization of the behaviour of a quartz crystal resonator fully immersed in a Newtonian liquid by impedance analysis. *Sensors Actuators A Phys.* **2011**, *167*, 317–326.
 47. Arnau, A. A Review of Interface Electronic Systems for AT-cut Quartz Crystal Microbalance Applications in Liquids. *Sensors* **2008**, *8*, 370–411.

48. Cassiède, M.; Paillol, J. H.; Pauly, J.; Daridon, J.-L. Electrical behaviour of AT-cut quartz crystal resonators as a function of overtone number. *Sensors Actuators A Phys.* **2010**, *159*, 174–183.
49. Wu, D. H.; Tsai, Y. J.; Yen, Y. T. Robust design of quartz crystal microbalance using finite element and Taguchi method. *Sensors Actuators B Chem.* **2003**, *92*, 337–344.
50. Cho, N.-J.; D'Amour, J. N.; Stalgren, J.; Knoll, W.; Kanazawa, K.; Frank, C. W. Quartz resonator signatures under Newtonian liquid loading for initial instrument check. *J. Colloid Interface Sci.* **2007**, *315*, 248–54.
51. Cernosek, R. W.; Martin, S. J.; Hillman, A. R.; Bandey, H. L. Comparison of lumped-element and transmission-line models for thickness-shear-mode quartz resonator sensors. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **1998**, *45*, 1399–1407.
52. Granstaff, V. E.; Martin, S. J. Characterization of a thickness-shear mode quartz resonator with multiple nonpiezoelectric layers. *J. Appl. Phys.* **1994**, *75*, 1319.
53. Rosenbaum, J. *Bulk Acoustic Wave Theory and Devices*; Artech Print on Demand: Boston, 1988.
54. Lucklum, R. The Δf – ΔR QCM technique: an approach to an advanced sensor signal interpretation. *Electrochim. Acta* **2000**, *45*, 3907–3916.
55. Wolfbeis, O. S. *Piezoelectric Sensors*; Steinem, C.; Janshoff, A., Eds.; Springer Series on Chemical Sensors and Biosensors; Springer Berlin Heidelberg, 2007; Vol. 5.
56. Chen, K.; Tan, Z.; Nie, L.; Yao, S. Principal component analysis applied to admittance spectra of a quartz-crystal microbalance in contact with a liquid phase. *Analyst* **1995**, *120*, 1885.
57. Ferrari, V.; Marioli, D.; Taroni, A. ACC oscillator for in-liquid quartz microbalance sensors. *Proc. IEEE Sensors 2003 (IEEE Cat. No.03CH37498)* **2003**, 849–854.
58. Ferreira, G. N. M.; da-Silva, A.-C.; Tomé, B. Acoustic wave biosensors: physical models and biological applications of quartz crystal microbalance. *Trends Biotechnol.* **2009**, *27*, 689–97.
59. Chaplin, M. rheology <http://www1.lsbu.ac.uk/water/rheology.html>.
60. DONDOS, A.; PAPANAGOPOULOS, D. Difference between the dynamic and static behaviour of polymers in dilute solutions: 1. The critical concentration. *Polymer (Guildf)*. **1995**, *36*, 365–368.
61. Br, U.; Meier, W.; Practices, B. *Product Design and Engineering*; Bröckel, U.; Meier, W.; Wagner, G., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2013.
62. Rao, M. A. *Rheology of Fluid and Semisolid Foods*; Food Engineering Series; Springer US, 2007; Vol. 53.
63. Br, U.; Meier, W.; Practices, B. *Product Design and Engineering*; Bröckel, U.; Meier, W.; Wagner, G., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2013.
64. Larson, R. G. The rheology of dilute solutions of flexible polymers: Progress and problems. *J. Rheol. (N. Y. N. Y.)* **2005**, *49*, 1–70.
65. Chen, Y.; Chou, P.; Cheng, C.; Chiang, C.; Wei, M.; Chuang, C. Microrheology of human synovial fluid of arthritis patients studied by diffusing wave spectroscopy. *J. Biophotonics* **2012**, 1–8.

66. Nwankwo, E.; Durning, C. Mechanical response of thickness-shear mode quartz-crystal resonators to linear viscoelastic fluids. *Sensors Actuators A Phys.* **1998**, *64*, 119–124.
67. Fang, J.; Zhu, T.; Sheng, J.; Jiang, Z.; Ma, Y. Thickness Dependent Effective Viscosity of a Polymer Solution near an Interface Probed by a Quartz Crystal Microbalance with Dissipation Method. *Sci. Rep.* **2015**, *5*, 8491.
68. Schurz, J.; Ribitsch, V. Rheology of synovial fluid. *Biorheology* **1987**, *24*, 385–399.
69. Oates, K. M. N.; Krause, W. E.; Jones, R. L.; Colby, R. H. Rheopexy of synovial fluid and protein aggregation. *J. R. Soc. Interface* **2005**, *3*, 167–174.
70. Teraoka, I. *Polymer Solutions*; John Wiley & Sons, Inc.: New York, USA, 2002; Vol. Chapter1:
71. Sunthar, P. Polymer rheology. *Rheol. Complex Fluids* **2010**, 171–191.
72. Li, D. Relaxation Time of the Viscoelastic Fluid. In *Encyclopedia of Microfluidics and Nanofluidics*; Li, D., Ed.; Springer US: Boston, MA, 2008; pp. 1781–1781.
73. Price, R. D.; Berry, M. G.; Navsaria, H. A. Hyaluronic acid: the scientific and clinical evidence. *J. Plast. Reconstr. Aesthetic Surg.* **2007**, *60*, 1110–1119.
74. Schanté, C. E.; Zuber, G.; Herlin, C.; Vandamme, T. F. Chemical modifications of hyaluronic acid for the synthesis of derivatives for a broad range of biomedical applications. *Carbohydr. Polym.* **2011**, *85*, 469–489.
75. Fraser, J. R. E. ; Laurent, T. C. ; Laurent, U. B. G. The nature of hyaluronan. *J. Intern. Med.* **1997**, *242*, 27–33.
76. Courtens, C. Characterization of Rheological and Mass Transport Properties of Hyaluronic Acid for Medical Use, GHENT UNIVERSITY, 2013.
77. Pitsillides, a a; Skerry, T. M.; Edwards, J. C. Joint immobilization reduces synovial fluid hyaluronan concentration and is accompanied by changes in the synovial intimal cell populations. *Rheumatology (Oxford)*. **1999**, *38*, 1108–12.
78. Laurent, T. C. Biochemistry of Hyaluronan. *Acta Otolaryngol.* **1987**, *104*, 7–24.
79. Doerner, S.; Schneider, T.; Schroder, J.; Hauptmann, P. Universal impedance spectrum analyzer for sensor applications. **2003**, *1*, 596–599 Vol.1.
80. Schröder, J.; Borngräber, R.; Eichelbaum, F.; Hauptmann, P. Advanced interface electronics and methods for QCM. *Sensors Actuators, A Phys.* **2002**, *97–98*, 543–547.
81. Calvo, E. J.; Etchenique, R.; Bartlett, P. N.; Singhal, K.; Santamaria, C. Quartz crystal impedance studies at 10 MHz of viscoelastic liquids and films. *Faraday Discuss.* **1997**, *107*, 141–157.
82. Rodahl, M.; Höök, F.; Krozer, A.; Brzezinski, P.; Kasemo, B. Quartz crystal microbalance setup for frequency and Q-factor measurements in gaseous and liquid environments. *Rev. Sci. Instrum.* **1995**, *66*, 3924.
83. Rodahl, M.; Kasemo, B. On the measurement of thin liquid overlayers with the quartz-crystal microbalance. *Sensors Actuators A Phys.* **1996**, *54*, 448–456.
84. Rodahl, M.; Kasemo, B. Frequency and dissipation-factor responses to localized liquid deposits on a QCM electrode. *Sensors Actuators B Chem.* **1996**, *37*, 111–116.

85. Biolin Scientific Holding AB Q-Sense <http://www.biolinscientific.com/q-sense/products/>.
86. Dixon, M. C. Quartz crystal microbalance with dissipation monitoring: Enabling real-time characterization of biological materials and their interactions. *J. Biomol. Tech.* **2008**, *19*, 151–158.
87. Rodahl, M.; Kasemo, B. A simple setup to simultaneously measure the resonant frequency and the absolute dissipation factor of a quartz crystal microbalance. *Rev. Sci. Instrum.* **1996**, *67*, 3238.
88. Rodahl, M.; Höök, F.; Kasemo, B. QCM Operation in Liquids: An Explanation of Measured Variations in Frequency and Q Factor with Liquid Conductivity. *Anal. Chem.* **1996**, *68*, 2219–27.
89. Fredriksson, C.; Kihlman, S.; Rodahl, M.; Kasemo, B. The Piezoelectric Quartz Crystal Mass and Dissipation Sensor: A Means of Studying Cell Adhesion. *Langmuir* **1998**, *14*, 248–251.
90. Reviakine, I.; Johannsmann, D.; Richter, R. P. Hearing What You Cannot See and Visualizing What You Hear: Interpreting Quartz Crystal Microbalance Data from Solvated Interfaces. *Anal. Chem.* **2011**, *83*, 8838–8848.
91. Fatissou, J.; Azari, F.; Tufenkji, N. Real-time QCM-D monitoring of cellular responses to different cytotoxic agents. *Biosens. Bioelectron.* **2011**, *26*, 3207–3212.
92. Su, X.-L.; Li, Y. A self-assembled monolayer-based piezoelectric immunosensor for rapid detection of Escherichia coli O157:H7. *Biosens. Bioelectron.* **2004**, *19*, 563–74.
93. Xi, J.; Chen, J. Y.; Garcia, M. P.; Penn, L. S. Biochips & Tissue Chips Quartz Crystal Microbalance in Cell Biology Studies. **2013**, 1–9.
94. Afonso, A. S.; Zanetti, B. F.; Santiago, A. C.; Henrique-Silva, F.; Mattoso, L. H. C.; Faria, R. C. QCM immunoassay for recombinant cysteine peptidase: A potential protein biomarker for diagnosis of citrus canker. *Talanta* **2013**, *104*, 193–197.
95. Lu, C. H.; Zhang, Y.; Tang, S. F.; Fang, Z. Bin; Yang, H. H.; Chen, X.; Chen, G. N. Sensing HIV related protein using epitope imprinted hydrophilic polymer coated quartz crystal microbalance. *Biosens. Bioelectron.* **2012**, *31*, 439–444.
96. Olanya, G.; Thormann, E.; Varga, I.; Makuska, R.; Claesson, P. M. Protein interactions with bottle-brush polymer layers: Effect of side chain and charge density ratio probed by QCM-D and AFM. *J. Colloid Interface Sci.* **2010**, *349*, 265–74.
97. Serro, A. P.; Degiampietro, K.; Colaço, R.; Saramago, B. Adsorption of albumin and sodium hyaluronate on UHMWPE: A QCM-D and AFM study. *Colloids Surfaces B Biointerfaces* **2010**, *78*, 1–7.
98. Chen, Q.; Tang, W.; Wang, D.; Wu, X.; Li, N.; Liu, F. Amplified QCM-D biosensor for protein based on aptamer-functionalized gold nanoparticles. *Biosens. Bioelectron.* **2010**, *26*, 575–579.
99. Michanek, A.; Kristen, N.; Höök, F.; Nylander, T.; Sparr, E. RNA and DNA interactions with zwitterionic and charged lipid membranes - A DSC and QCM-D study. *Biochim. Biophys. Acta - Biomembr.* **2010**, *1798*, 829–838.
100. Tsortos, A.; Papadakis, G.; Gizeli, E. Shear acoustic wave biosensor for detecting DNA intrinsic viscosity and conformation: a study with QCM-D. *Biosens. Bioelectron.* **2008**, *24*, 842–7.

101. Zhou, X. C.; Huang, L. Q.; Li, S. F. Microgravimetric DNA sensor based on quartz crystal microbalance: comparison of oligonucleotide immobilization methods and the application in genetic diagnosis. *Biosens. Bioelectron.* **2001**, *16*, 85–95.
102. Hao, R. Z.; Song, H. Bin; Zuo, G. M.; Yang, R. F.; Wei, H. P.; Wang, D. B.; Cui, Z. Q.; Zhang, Z.; Cheng, Z. X.; Zhang, X. E. DNA probe functionalized QCM biosensor based on gold nanoparticle amplification for *Bacillus anthracis* detection. *Biosens. Bioelectron.* **2011**, *26*, 3398–3404.
103. Wang, D.; Chen, G.; Wang, H.; Tang, W.; Pan, W.; Li, N.; Liu, F. A reusable quartz crystal microbalance biosensor for highly specific detection of single-base DNA mutation. *Biosens. Bioelectron.* **2013**, *48*, 276–80.
104. García-Martínez, G.; Bustabad, E. A.; Perrot, H.; Gabrielli, C.; Bucur, B.; Lazerges, M.; Rose, D.; Rodríguez-Pardo, L.; Fariña, J.; Compère, C.; Arnau, A. Development of a mass sensitive quartz crystal microbalance (QCM)-based DNA biosensor using a 50 MHz electronic oscillator circuit. *Sensors (Basel)*. **2011**, *11*, 7656–64.
105. Eris, G.; Bozkurt, A. A.; Sunol, A.; Jonáš, A.; Kiraz, A.; Alaca, B. E.; Erkey, C. Determination of viscosity and density of fluids using frequency response of microcantilevers. *J. Supercrit. Fluids* **2015**, *105*, 179–185.
106. Jakoby, B.; Beigelbeck, R.; Keplinger, F.; Lucklum, F.; Niedermayer, A.; Reichel, E. K.; Riesch, C.; Voglhuber-Brunnmaier, T.; Weiss, B. Miniaturized sensors for the viscosity and density of liquids--performance and issues. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **2010**, *57*, 111–120.
107. Dunér, G.; Thormann, E.; Dédinaite, A. Quartz Crystal Microbalance with Dissipation (QCM-D) studies of the viscoelastic response from a continuously growing grafted polyelectrolyte layer. *J. Colloid Interface Sci.* **2013**, *408*, 229–234.
108. Sweity, A.; Ying, W.; Ali-Shtayeh, M. S.; Yang, F.; Bick, A.; Oron, G.; Herzberg, M. Relation between EPS adherence, viscoelastic properties, and MBR operation: Biofouling study with QCM-D. *Water Res.* **2011**, *45*, 6430–6440.
109. Jakoby, B.; Art, G.; Bastemeijer, J. Novel analog readout electronics for microacoustic thickness shear-mode sensors. *Sensors Journal, IEEE* **2005**, *5*, 1106–1111.
110. Yu, G. Y. Magnetic Quartz Crystal Microbalance, 2008.
111. Vavra, K. C.; Yu, G.; Josowicz, M.; Janata, J. Magnetic quartz crystal microbalance: Magneto-acoustic parameters. *J. Appl. Phys.* **2011**, *110*, 13905.
112. Kim, G. H.; Rand, a G.; Letcher, S. V Impedance characterization of a piezoelectric immunosensor. Part I: antibody coating and buffer solution. *Biosens. Bioelectron.* **2003**, *18*, 83–9.
113. Johannsmann, D.; Mathauer, K.; Wegner, G.; Knoll, W. Viscoelastic properties of thin films probed with a quartz-crystal resonator. *Phys. Rev. B. Condens. Matter* **1992**, *46*, 7808–7815.
114. Wessendorf, K. K. O. The Lever oscillator for use in high resistance resonator applications. *1993 IEEE Int. Freq. Control Symp.* **1993**, 711–717.
115. Wessendorf, K. O. The active-bridge oscillator for use with liquid loaded QCM sensors. **2001**, 400–407.
116. Papez, V.; Papezova, S. Bridge symmetric crystal oscillator. *2011 10th Int. Conf.*

Telecommun. Mod. Satell. Cable Broadcast. Serv. TELSIKS 2011 - Proc. Pap. **2011**, 2, 597–600.

117. Schweyer, M.; Hilton, J.; Munson, J. E.; Andle, J. C.; Hammond, J. M.; Lec, R. M. A novel monolithic piezoelectric sensor. In *Proceedings of International Frequency Control Symposium*; IEEE, 1997; pp. 32–40.

118. Borngräber, R.; Schröder, J.; Lucklum, R.; Hauptmann, P. Is an oscillator-based measurement adequate in a liquid environment? *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **2002**, 49, 1254–1259.

119. Rodriguez-Pardo, L.; Farina, J.; Gabrielli, C.; Perrot, H.; Brendel, R. Miller oscillators for high sensitivity quartz crystal microbalance sensors in damping media. **2004**, 0, 806–812.

120. Rodriguez-Pardo, L.; Fariña, J.; Gabrielli, C.; Perrot, H.; Brendel, R. Design considerations of miller oscillators for high-sensitivity QCM sensors in damping media. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **2007**, 54, 1965–1976.

121. Ferrari, M.; Ferrari, V.; Marioli, D.; Taroni, A.; Suman, M.; Dalcanele, E. In-liquid sensing of chemical compounds by QCM sensors coupled with high-accuracy ACC oscillator. *IEEE Trans. Instrum. Meas.* **2006**, 55, 828–834.

122. Tuantranont, A.; Wisitsora-at, A.; Sritongkham, P.; Jaruwongrungrongsee, K. A review of monolithic multichannel quartz crystal microbalance: a review. *Anal. Chim. Acta* **2011**, 687, 114–28.

123. Sankaranarayanan, S. K. R. S.; Singh, R.; Bhethanabotla, V. Computational design of quartz crystal nanobalance for uniform sensitivity distribution. In *2010 IEEE Sensors*; IEEE, 2010; pp. 1883–1886.

124. Sun, H.; Lu, P.; Zhang, P.; Chen, H. Dynamic analysis of AT-cut quartz resonators with ANSYS. *Sensors, 2004. Proc. ...* **2004**, 1, 95–98.

125. Ji, J.; Oigawa, H.; Chen, H. H.; Zhao, M.; Ueda, T. Size optimization for high frequency quartz resonator using finite element vibration analysis. *2011 IEEE SENSORS Proc.* **2011**, 1652–1655.

126. Lu, F.; Lee, H. P.; Lu, P.; Lim, S. P. Finite element analysis of interference for the laterally coupled quartz crystal microbalances. *Sensors Actuators A Phys.* **2005**, 119, 90–99.

127. Reinhold Ludwig, P. B. RF Circuit Design - Theory and Applications 2000, 201–253.

128. Statek The quartz crystal model and its frequencies. *Tech. Note 32 I*, 1–9.

129. Auge, J.; Hauptmann, P.; Hartmann, J. New design for QCM sensors in liquids. *Sensors Actuators B ...* **1995**, 24, 43–48.

130. Talib, Z.; Baba, Z.; Kurosawa, S.; Sidek, H. A. A.; Kassim, A.; Yunus, W. M. M. Frequency Behavior of a Quartz Crystal Microbalance (Qcm) in Contact with Selected Solutions. *Am. J. Appl. Sci.* **2006**, 3, 1853–1858.

131. Overview - Arthritis - Mayo Clinic <http://www.mayoclinic.org/diseases-conditions/arthritis/home/ovc-20168903>.

132. What is arthritis <http://www.arthritis.org/about-arthritis/understanding-arthritis/what-is-arthritis.php>.

133. Rojas, C. Estudio del líquido sinovial. *Guías procedimientos en Reumatol.* 2011, 41–47.

134. DECKER, B.; McGUICKIN, W. F.; McKENZIE, B. F.; SLOCUMB, C. H. Concentration of hyaluronic acid in synovial fluid. *Clin. Chem.* **1959**, 5, 465–469.
135. Balazs, E. A. The Physical Properties of Synovial Fluid and the Special Role of Hyaluronic Acid. *Disord. Knee* **1974**, 2, 63–75.
136. Corporation, worthington biochemical Ovalbumin <http://www.worthington-biochem.com/oa/default.html>.
137. Cardinaels, R.; Velde, J. Van De; Mathues, W.; Liedekerke, P. Van; Moldenaers, P. A rheological characterisation of liquid egg albumen. *Proc. Insid. Food Symp. 9-12 April 2013, Leuven, Belgium* **2013**, 1–6.
138. Bird, R. B.; Armstrong, C. R.; Hassager, O. *Dynamics of polymeric liquids, second edition, volume 1*; Wiley-Interscience: New York, NY, USA, 1987; Vol. 25.

APPENDIX A: Maxwell Constitutive Equations

Equations (12) and (13) emerge from the Maxwell constitutive Equation [138]:

$$\tau + \lambda_1 \frac{\partial \tau}{\partial t} = -\eta_0 \dot{\gamma} \quad (\text{A1})$$

where: $\dot{\gamma}$ is the rate of strain tensor, η_0 is the zero shear rate viscosity and λ_1 is the relaxation time.

Solving for τ , integrating:

$$\tau(t) = \frac{-\int_{-\infty}^t \left(\frac{\eta_0}{\lambda_1} \dot{\gamma}(t')\right) e^{t'/\lambda_1} dt'}{e^{t/\lambda_1}} + k e^{-t/\lambda_1} \quad (\text{A2})$$

where primes have been added to t 's in the integrand in order to avoid confusion. k is the integration constant. If we prescribe that the stress in the fluid is finite at $t = -\infty$, we must set k on zero.

Applying L'Hopital's rules in Equation (A2):

$$\lim_{t \rightarrow -\infty} \tau(t) = \lim_{t \rightarrow -\infty} \left(-\frac{\left(\frac{\eta_0}{\lambda_1} \dot{\gamma}(t)\right) e^{t/\lambda_1}}{\frac{1}{\lambda_1} e^{t/\lambda_1}} \right) = -\eta_0 \dot{\gamma}(-\infty) \quad (\text{A3})$$

Therefore, if $\dot{\gamma}(-\infty)$ is finite, the stress is also finite at $t = -\infty$. Consequently, the Maxwell constitutive equation can be written in the form:

$$\tau(t) = -\int_{-\infty}^t \left(\frac{\eta_0}{\lambda_1} e^{-(t-t')/\lambda_1} \right) \dot{\gamma}(t') dt' \quad (\text{A4})$$

The quantity in brackets is called the relaxation modulus for Maxwell fluid. This expression shows that the stress at the present time t depends on the rate of strain at time t as well as on the rate of strain at all past times t' with a weighting factor (relaxation modulus) that decays exponentially as one goes backwards in time.

Taking into account:

$$\gamma_{yx}(t_0, t) = \int_{t_0}^t \dot{\gamma}_{yx}(t') dt' \quad (\text{A5})$$

Then:

$$\frac{\partial \gamma}{\partial t'} = \dot{\gamma}(t) \quad (\text{A6})$$

$$\gamma_{yx}(t, t') = \int_t^{t'} \dot{\gamma}(t'') dt'' \quad (\text{A7})$$

Now, integrating by parts $\tau(t)$:

$$\tau(t) = + \int_{-\infty}^t \left(\frac{\eta_0}{\lambda_1^2} e^{-(t-t')/\lambda_1} \right) \dot{\gamma}(t, t') dt' \quad (\text{A8})$$

The quantity in brackets is called memory function for Maxwell fluid. In this form the Maxwell model states that the stress at the present time t depends on the history of the strain for all past times $-\infty < t' < t$.

Now, considering the Equation (1) as a sums of partial stresses (Generalized Maxwell Model):

$$\tau(t) = \sum_{k=1}^{\infty} \tau_k(t) \quad (\text{A9})$$

$$\tau_k + \lambda_k \frac{\partial \tau_k}{\partial t} = -\eta_k \dot{\gamma} \quad (\text{A10})$$

The shear stress can be written as:

$$\tau(t) = - \int_{-\infty}^t \left[\sum_{k=1}^{\infty} \frac{\eta_k}{\lambda_k} e^{-(t-t')/\lambda_k} \right] \dot{\gamma}(t') dt' \quad (\text{A11})$$

$$\tau(t) = + \int_{-\infty}^t \left[\sum_{k=1}^{\infty} \frac{\eta_k}{\lambda_k^2} e^{-(t-t')/\lambda_k} \right] \dot{\gamma}(t') dt' \quad (\text{A12})$$

where:

$$\eta_k = \frac{\lambda_k}{\sum_k \lambda_k} \quad (\text{A13})$$

$$\lambda_k = \frac{\lambda}{k^\alpha} \quad (\text{A14})$$

λ is a constant time and α is a dimensionless quantity which describes the slope of η' versus ω on a log-log plot at high ω .

The Rouse molecular theory for dilute polymer solutions gives $\alpha = 2$. Additionally, in the general linear viscoelastic model, the Equations (A11) and (A12) can be written as:

$$\tau = - \int_{-\infty}^t G(t-t') \dot{\gamma}(t') dt' \quad (\text{A15})$$

$$\tau = + \int_{-\infty}^t M(t-t') \dot{\gamma}(t, t') dt' \quad (\text{A16})$$

where the memory function M is the derivative of the relaxation modulus G .

Now, supposing a small amplitude oscillatory motion for the fluid:

$$\dot{\gamma}_{yx}(t) = \dot{\gamma}_{yx}^0 \cos(\omega t) \quad (\text{A17})$$

$\dot{\gamma}_{yx}^0$ is real and positive.

Resolving Equation (A15) taking into account Equation (A17):

$$\tau_{yx} = - \int_{-\infty}^t G(t-t') \dot{\gamma}_{yx}^0 \cos(\omega t') dt' \quad (\text{A18})$$

$$\tau_{yx} = -\dot{\gamma}_{yx}^0 \int_0^\infty G(s) \cos(\omega(t-s)) ds \quad (\text{A19})$$

$$\begin{aligned} \tau_{yx} = & - \left[\int_0^\infty G(s) \cos(\omega s) ds \right] \dot{\gamma}_{yx}^0 \cos(\omega t) \\ & - \left[\int_0^\infty G(s) \sin(\omega s) ds \right] \dot{\gamma}_{yx}^0 \sin(\omega t) \end{aligned} \quad (\text{A20})$$

Considering:

$$\tau_{yx} = -\eta' \dot{\gamma}_{yx}^0 \cos(\omega t) - \eta'' \dot{\gamma}_{yx}^0 \sin(\omega t) \quad (\text{A21})$$

Then:

$$\eta' = \int_0^\infty G(s) \cos(\omega s) ds \quad (\text{A22})$$

$$\eta'' = \int_0^\infty G(s) \sin(\omega s) ds \quad (\text{A23})$$

Now, replacing the Equation (A11) in Equation (A22):

$$\eta' = \int_0^\infty \left[\sum_{k=1}^\infty \frac{\eta_k}{\lambda_k} e^{-s/\lambda_k} \right] \cos(\omega s) ds \quad (\text{A24})$$

Solving for integration by parts two times:

$$\begin{aligned} \int_0^\infty \left[\sum_{k=1}^\infty \frac{\eta_k}{\lambda_k} e^{-s/\lambda_k} \right] \cos(\omega s) ds &= \sum_{k=1}^\infty \frac{\eta_k}{\lambda_k^2} \frac{1}{\omega^2} - \\ &\sum_{k=1}^\infty \left(\frac{1}{\lambda_k^2 \omega^2} \right) * \left[\int_0^\infty \left[\sum_{k=1}^\infty \frac{\eta_k}{\lambda_k} e^{-s/\lambda_k} \right] \cos(\omega s) ds \right] \end{aligned} \quad (\text{A25})$$

This equation can be written as:

$$\eta' = \sum_{k=1}^\infty \frac{\eta_k}{\lambda_k^2} \frac{1}{\omega^2} - \sum_{k=1}^\infty \left(\frac{1}{\lambda_k^2 \omega^2} \right) * \eta' \quad (\text{A26})$$

Solving for η' :

$$\eta' = \sum_{k=1}^\infty \frac{\eta_k}{1 + (\lambda_k \omega)^2} \quad (\text{A27})$$

Solving in similar way for η'' :

$$\eta'' = \sum_{k=1}^\infty \frac{\eta_k \lambda_k \omega}{1 + (\lambda_k \omega)^2} \quad (\text{A28})$$

In addition, using the expressions (A13) and (A14) in Equations (A27) and (A28), these results become:

$$\frac{\eta'}{\eta_0} = \frac{1}{\zeta(\alpha)} \sum_{k=1}^\infty \frac{k^\alpha}{k^{2\alpha} + (\lambda \omega)^2} \quad (\text{A29})$$

$$\frac{\eta''}{\eta_0} = \frac{\lambda \omega}{\zeta(\alpha)} \sum_{k=1}^\infty \frac{1}{k^{2\alpha} + (\lambda \omega)^2} \quad (\text{A30})$$

Changing λ to τ_0 and α to ν . Equations (A29) and (A30) are the same Equations (13) and (14).

In addition, the stress can be defined as:

$$\tau_{yx} = \tau_{yx}^0 \sin(\omega t + \delta) \quad (\text{A31})$$

Where δ is the phase difference between the stress and the deformation.

$$\tau_{yx} = \tau_{yx}^0 (\cos(\omega t) \sin(\delta) + \cos(\delta) \sin(\omega t)) \quad (\text{A32})$$

Linking A32 to A21:

$$\eta' = \frac{\tau^0_{yx}}{\dot{\gamma}^0_{yx}} \sin(\delta) \quad (\text{A33})$$

$$\eta'' = \frac{\tau^0_{yx}}{\dot{\gamma}^0_{yx}} \cos(\delta) \quad (\text{A34})$$

$$\tan(\delta) = \frac{\eta'}{\eta''} \quad (\text{A35})$$