Chapter 8. Progress towards detectors for the determination of enantiomeric excess

8.1. Introduction

One of the most fascinating questions in prebiotic chemistry is that of the origin of homochirality. All life on Earth is based on the L-amino acids and D-sugars. There is no particular reason for these choices; the combination of the D-amino acids and L-sugars should work just as well. The intriguing facet of this characteristic of life is the idea that life is unlikely to evolve from a racemic mixture of compounds. Homochirality in the amino acids that led to the first peptides and proteins would have been necessary to insure that the polymer would fold into the proper secondary and tertiary conformations to function properly. [Ehrenfreund, et al., 2004]

There have been many theories as to how homochirality could have developed prior to the origin of life. [Podlech, 2001]; [Siegel, 1998] The most likely candidates seem to be some form of amplification of an initial small excess, or the action of polarized light to selectively destroy one enantiomer over the other. [Avalos, *et al.*, 2000] Of course, the actual events that led to homochirality on Earth are impossible to determine. As well, experiments that replicate prebiotic conditions are impractical, given the large volumes and long timescales that would be necessary. As discussed in Chapter 2, these experiments have probably been performed on Titan naturally over the course of millions of years. Examination of the frozen melt pools of Titan, paying special attention

to the enantiomeric excess of any chiral compounds, could provide valuable insights into the origin of homochirality and the origin of life.

There are a great variety of methods for the determination of enantiomeric excess [Schreier*, et al.*, 1995], but few are suitable for spaceflight applications. Polarimetric methods are not very sensitive. NMR techniques require the bulk of an NMR magnet. The most developed of the chiral separation techniques, gas chromatography, is suitable for spaceflight, but unsuitable for the analysis of complex mixtures, such as would be found on Titan. While GC-MS with a chiral column would separate the components of a complex mixture as well as separate any chiral compounds that are present, the resulting chromatogram will likely be too complex to be easily interpretable.

A different approach to this problem is outlined below. Instead of a chiral column, the gas chromatograph is equipped with a standard, non-chiral column. Two detectors are used. The second detector is a standard mass spectrometer detector to enable identification of the compounds. Prior to the mass spectrometer is an enantiomeric excess detector. Such a detector would be nondestructive, and would signal when an enantiomeric excess is detected. Racemic chiral compounds passing through the detector would not result in a signal.

This chapter describes an approach to the development of a detector for the determination of enantiomeric excess. The sensor is composed of two quartz crystal microbalances (QCMs). [Lu and Czanderna, 1984] A QCM is a thin plate of quartz, with a metal electrode plated on each side. When placed in the proper circuit, the QCM resonates at a specific frequency proportional to its mass. The relationship between frequency and mass is given in equation (8.1), where Δf(Hz) is the change in frequency, f

is the fundamental frequency of the crystal, d_q is the density of quartz (2.65 g cm^{-3}), N is the frequency constant for an AT-cut crystal (1.670 x 10^5 cm Hz), Δm (g cm^{-2}) is the added mass per unit area, and m (g cm^{-2}) is the mass per unit area of quartz [Edmonds, 1988]. In order to create a chemical sensor, the QCM is coated with an absorbent film (either selective or non-selective). When the coated QCM is exposed to analyte, the film absorb some of the analyte, the mass of the device increases, and the frequency drops. QCMs are capable of mass resolution in the nanogram range.

$$\Delta f = - \{f^2 / d_q N\} \{\Delta m / [1 + \Delta m / m]\} \quad (8.1)$$

To function as enantiomeric excess detector, one crystal is coated with an enantiomeric film that selectively adsorbs one enantiomer of an analyte in greater quantity than the other. The other crystal is coated with a film of the opposite enantiomer. The output of the two crystal oscillators is subtracted, and this final frequency is monitored. In this configuration, the difference frequency is a direct measure of the enantiomeric excess.

Enantiomerically selective QCMs have been developed by a number of other groups, although mainly for use in liquids, not gases. Bodenhoffer described a sensor based on the chiral GC phase Chirasil-Val that was able to distinguish between the enantiomers of methyl lactate and certain amino acid derivatives. [Bodenhofer*, et al.*, 1997a]; [Bodenhofer*, et al.*, 1997b] However, a dual QCM sensor that utilizes a pair of enantiomeric sensors was not developed.

We describe progress in the development of the kind of sensor described above. The gold electrode of the QCM is coated with a self-assembled monolayer that is derivatized with a chiral selector. The response of the sensor to the enantiomers of methyl lactate is presented. As well, we also present data for a similar piezoelectric sensor system based on a monolayer derivatized surface acoustic wave (SAW) device. SAW sensors are similar to QCMs in that both are piezoelectric mass sensors [Edmonds, 1988], but SAWs are several orders of magnitude more sensitive, smaller and would result in an improved sensor system.

8.2. Experimental

A block diagram of the QCM apparatus is shown in Figure 8.1. Two 15 MHz quartz crystal microbalances (International Crystal Manufacturing, Oklahoma City, OK) are held in an aluminum cell with an internal volume of approximately 10 mL. Analyte vapor is delivered to the cell with a Model 1010 Precision Gas Diluter (Custom Sensor Solutions, Inc., Oro Valley, AZ). Stock vapor samples were prepared in Tedlar bags for delivery to the diluter. The dilution gas used was nitrogen. Each crystal was connected to a lab-built Colpitts oscillator. The circuit diagram for the oscillators is shown in Figure 2. The output of both oscillators is fed to an SLB-1 frequency mixer (Mini-Circuits, Inc., Brooklyn, NY), and its output is fed to an Agilent 53131A frequency counter. The difference frequency determined by the counter is recorded once each second using custom software written with Labview (National Instruments, Austin , TX).

Experiments with SAW devices utilized a commercial dual crystal SAW sensor system obtained from Microsensor Systems, Inc. (Bowling Green, KY). The system

integrates two 250 MHz SAWs in a small brass chamber directly mounted on a circuit board. The circuitry oscillates both SAWs and outputs their difference frequency. Analyte vapor was delivered to the SAWs using the gas dilution system described above.

The following procedure was used to apply a monolayer on the surface of the gold electrode of the QCM (Scheme 8.1). The QCM was cleaned by applying a drop of piranha solution (3:1 concentrated H_2SO_4: 30% H_2O_2) to each side for 1 minute, followed by a thorough rinse with deionized water. (Caution! Piranha solution is a strong oxidizer and extremely corrosive, and can react violently with organics. Use with care.) The QCM was then immersed in a 5 mM solution of 16-mercaptohexadecanoic acid in methanol for 2 hours. A decanethiol monolayer was applied to some crystals by using a 5 mM solution of decanethiol.

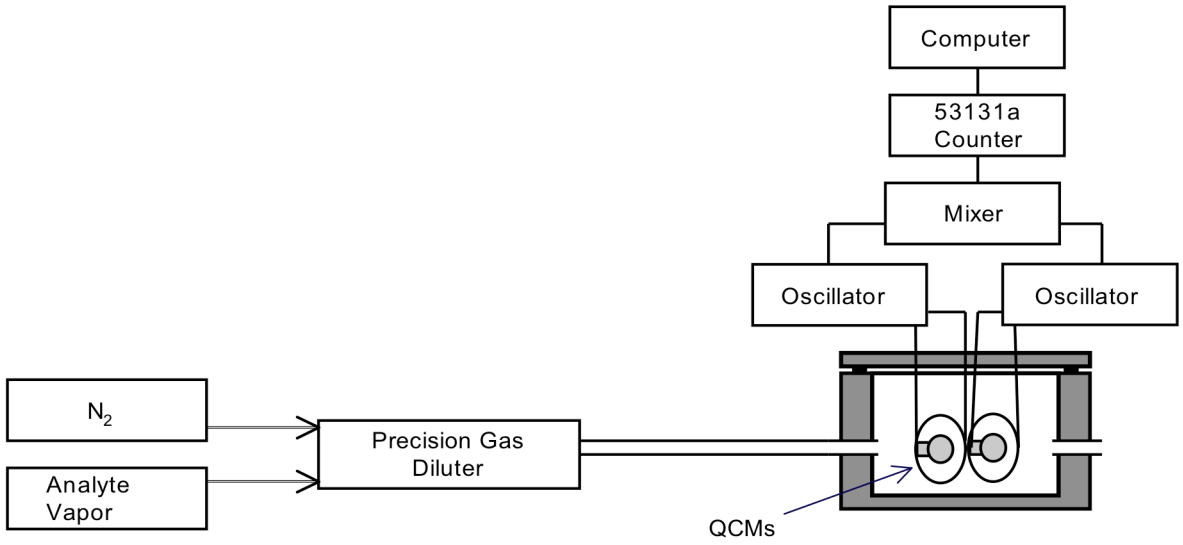


Figure 8.1. Block diagram of the QCM apparatus.

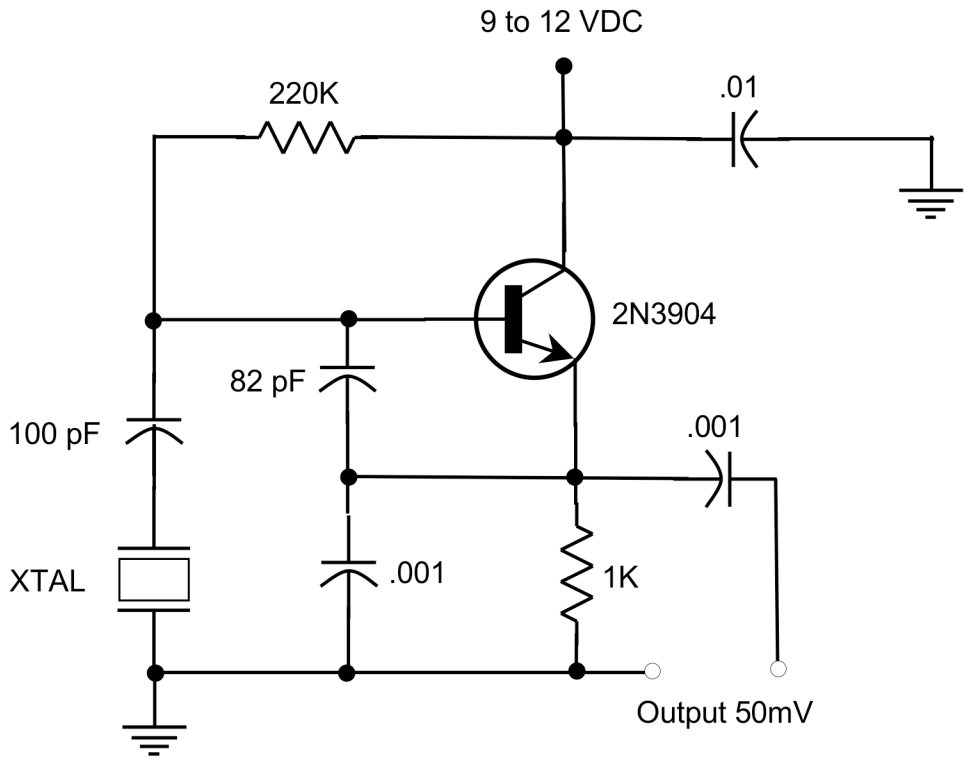
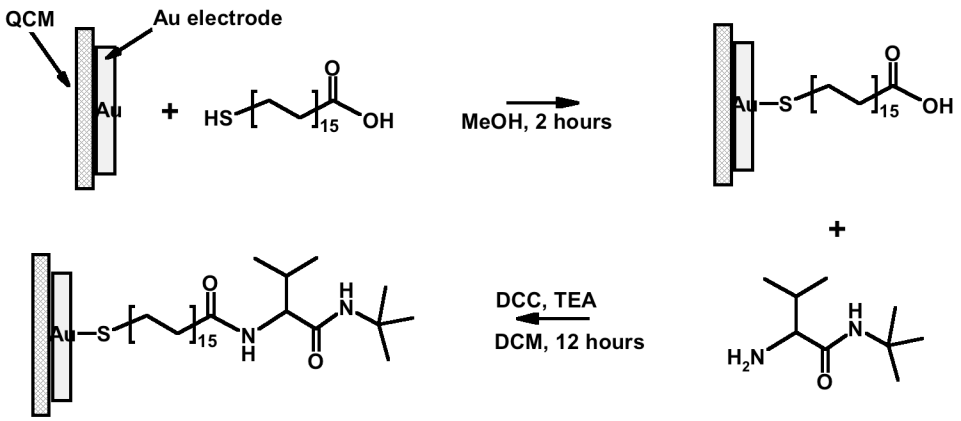


Figure 8.2. Colpitts oscillator used to drive the QCMs.

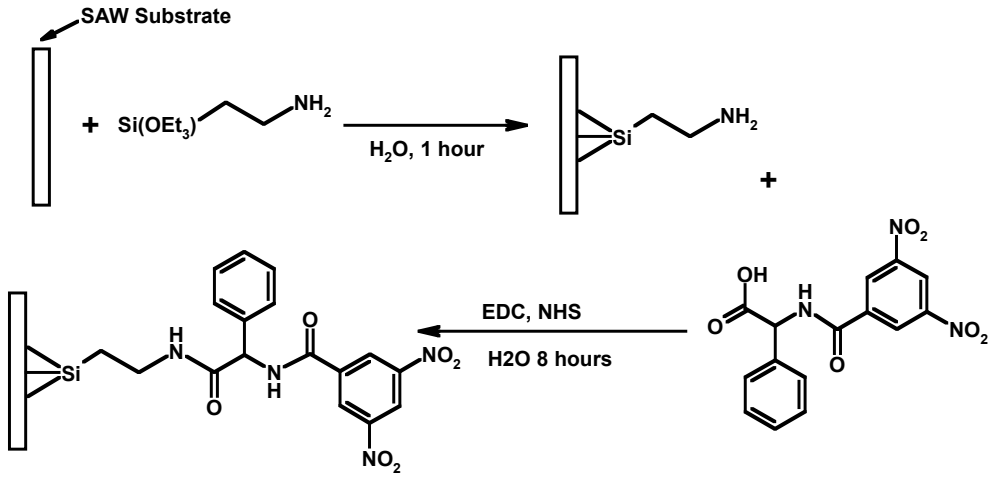
The crystal is then rinsed with methanol, and immersed in a solution of L-valine-tert-butylamide, triethylamine, and dicyclohexylcarbodiimide in dichloromethane for 12 hours. The synthetic procedure is illustrated in Scheme 8.1.

SAW devices require a different derivatization strategy, illustrated in Scheme 8.2, since the surface to be derivatized is quartz, rather than gold. The SAW device is cleaned with piranha solution for 2 minutes, then rinsed with water and dried under a stream of nitrogen. The SAW is then placed in a 10% solution of (3-aminopropyl)triethoxysilane for 6 hours. The SAW is rinsed with actone, and then placed in a solution of 19.6 mg 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 32.8 mg N-

hydroxysuccinimide (NHS), and 31.2 mg (R)-(3,5-dinitrobenzoyl-α-phenylglycine) in 10 mL of water for 1 hour. The SAW is rinsed with water and dried under a stream of nitrogen.



Scheme 8.1. Synthesis of an L-valine-tert-butylamide monolayer on a QCM electrode.



Scheme 8.2. Synthesis of an N-[(3,5-dinitrobenzoyl)phenyl]glycine monolayer on a SAW substrate.

8.3. Results and discussion

Figure 8.3 is a graph of the change in difference frequency of the crystal pair on exposure to each enantiomer of methyl lactate vapor, at several different concentrations. In this experiment, a crystal with an L-valine tert-butyl amide derivatized monolayer was paired with a decanethiol monolayer crystal. This allows for non-specific interactions, and the effects of temperature variations, to be canceled out. The response to the S enantiomer of methyl lactate is greater than the response to the R enantiomer. Valine tert-butyl amide was chosen as the chiral selector because its efficacy has been proven through its use in the popular GC stationary phase Chirasil-Val.

Experiments in which one crystal was derivatized with L-valine-tert-butylamide, and the other D-valine-tert-butyl amide yielded inconsistent results. This is likely due to difficulties in creating monolayers of the different enantiomers that are sufficiently identical to yield the proper differential response. More consistent monolayers could be created by preparing a chiral selector that incorporates a thiol moiety. The chiral monolayer would then be prepared in a single step, and the difficulties that arise from multiple reaction steps on monolayers could be avoided.

Figure 8.4 is graph of the change in difference frequency for the SAW sensor pair. One SAW is coated with a derivatized monolayer of N-[(3,5-dinitrobenzoyl)phenyl]glycine, and the other SAW is left uncoated, to serve as a reference. While enatioselectivity was not observed, we can see the much greater sensitivity of the SAW sensors. A 16 ppm exposure results in a 450 Hz frequency shift on the SAW, while a similar exposure of the QCM sensor results in a 3 Hz shift.

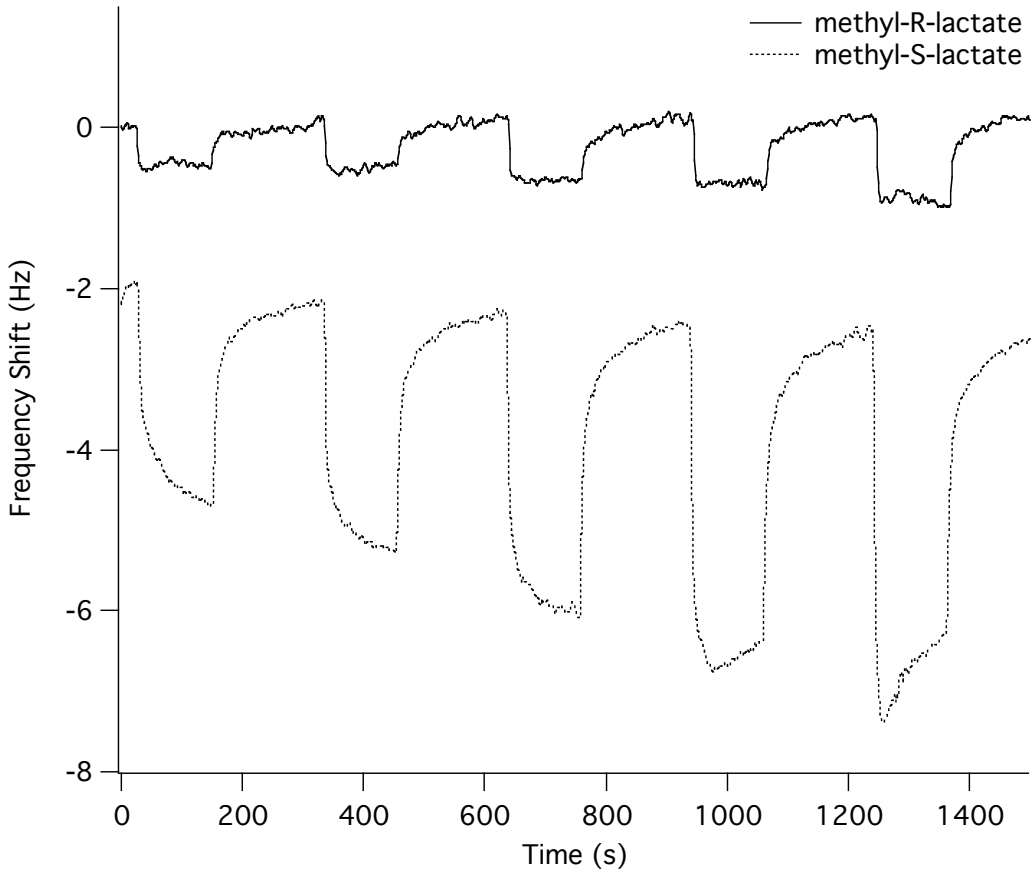


Figure 8.3. Responce of the QCM sensor to methyl lactate vapor. Concentrations are 16, 32, 64, 128, and 256 ppm, in that order.

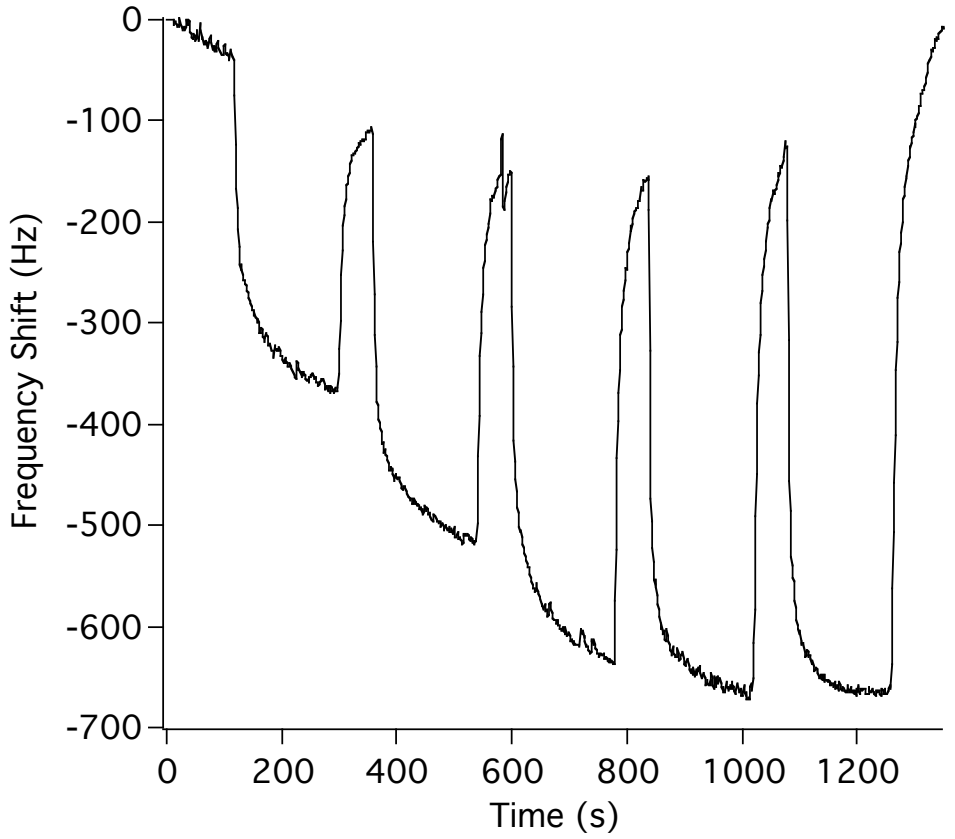


Figure 8.4. Response of a N-[(3,5-dinitrobenzoyl)phenyl]glycine derivatized SAW sensor to R-methyl lactate vapor. Concentrations are 3.9, 7.8, 11.7, 15.6 and 19.5 ppm.

8.4. Conclusions

Enantiomerically derivatized monolayers on the electrodes of a quartz crystal microbalance are capable of distinguishing between the enantiomers of methyl lactate. The sensor utilizes an L-valine tert-butyl amide selector. Unfortunately, the synthesis of the monolayer on the QCM electrode is too inconsistent to allow the construction of a sensor composed of two enantiomeric QCMs. Such a sensor would allow for the direct determination of enantiomeric excess.

This work represents progress towards the development of such a sensor. In its final incarnation, surface acoustic wave sensors, instead of QCMs may be used. The greater sensitivity of these devices (as shown here) would allow for more precise determination of enantiomeric excess, and the analysis of smaller quantities of material.

8.5. References

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