

5.8: FTIR on Membranes

Fourier Transform Infrared (FTIR) Spectroscopy is a widespread, relatively cheap technique for studying the structure of compounds through chemical bond vibrations. Since there are already pages dedicated to FTIR and Attenuated **Total Reflectance Fourier Transform Infrared Spectroscopy** (ATR-FTIR) this page will not go into the details of the theory associated with these techniques, but will instead describe these techniques in relation to lipid membranes.

Brief Overview of FTIR

This technique uses unpolarized radiation that is most often emitted using an interferometer. Since each chemical bond vibrates at a specific frequency absorption occurs when radiation at the same frequency is encountered. An interferometer is often the source of radiation due to its ability to scan through radiation frequencies continuously. The output of the instrument is an interferogram which is Fourier transformed into an output of percent transmittance versus wavenumber(cm^{-1}). Most IR spectrometers emit wavenumbers in the range of 400cm^{-1} to 4000cm^{-1} . Biomembranes are good candidates for FTIR because most phospholipids have infrared active bonds on the headgroup, interfacial region and hydrophobic region(Ruthven N.A.H. and Lewis, 1998)[1]. Since FTIR can provide information on the degrees of freedom within a molecule it is useful in determining how physically constrained a sample of molecules are. Two major uses of FTIR applied to lipid membranes are to study the fine structure of the lipid components and the phase transition properties of a lipid membrane.

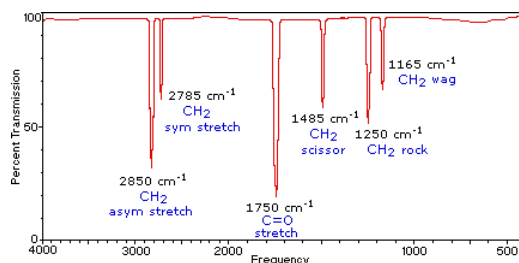


Figure 5.8.1: IR spectrum of Formaldehyde. (CC BY-SA-ND; William Reusch). A table of IR absorbance's can also be found at www2.chemistry.msu.edu/faculty/reusch/virttxtjml/Spectrpy/InfraRed/infrared.htm

Structure

Liquid Phase Phospholipid Bilayers

Liquid Phase membranes are characterized by the ability to rapidly move in the plane of the membrane and occasionally flip between the two membranes. The movement between two membranes is called a flip-flop. The structure of lamellar liquid-crystalline lipid bilayers are of special interest to the biological and biomedical sciences because it is the only lipid phase which has been demonstrated to exist in adapted biological membranes (McElhaney, 1984)[2]. Since this phase also has the most vibrational freedom it also produces broader bands on the IR spectrum. Despite this drawback FTIR can still be used on liquid-crystalline samples to tell us about the hydration states and hydrogen bonding of lipids using the phosphate absorbance of a phospholipid. The hydrocarbon chain methylene groups of liquid crystalline produce absorbance's that can be used to measure conformational disorder of the chain due to methylene group rocking in vibrational modes (Snyder and Poore, 1973)[3].

Gel-Phospholipid Bilayers

Gel phase, also referred to as solid phase, is characterized by reduced lateral mobility and the loss of the ability to flip-flop. Due to the decrease in mobility and conformational freedom the FTIR spectrum of gel phase membranes produce sharper bands resulting in higher resolution that is useful for structural studies, in particular relating to the hydrocarbon chain and if temperature is reduced enough eventually peaks from headgroups become sharper. FTIR on gel phase lipid bilayers is what demonstrated that some, and perhaps all, lipid membranes squeeze water out of the polar/apolar interfaces when the hydrocarbon tails undertake the mixed-interdigitated form upon cooling (Lewis and McElhaney 1993)[4]. An important conformational difference between liquid and gel the phase is that in gel the phase all methylene groups of the hydrocarbon chain are in the trans conformation. Trans conformation can be seen by the disappearance of methylene rocking in the gel phase. Some have taken to using this property a sign for hydrocarbon chain-melting phase transition (Chia and Mendelsohn, 1992; Chia et al., 1993)[5][6].

Crystalline Phospholipid Bilayers

Crystalline phase lipids are fixed in their position and contain the lowest level of conformational freedom which produces the sharpest peaks with the finest level of detail, but collecting this type of information may require specialized IR techniques using isotopically labeled materials(Lewis and McElhaney, 1996)[7]. Although the number of phospholipids measured at these temperatures is limited, one property that has been shown for phosphatidyl ethanolamine(PE) bilayers is that headgroup-headgroup interactions persist to very long chain lengths(Lewis and McElhaney, 1993a)[8].

Phase Transitions

A major use of FTIR on phospholipid bilayers is to study phase transitions of various types of lipids. To do this a table of common IR bands are used to characterize the phase transition(Lewis, R. N. A. H. and McElhaney, R. N. 1996)[9]. Although phase transitions in samples are usually quite complete, biologically speaking it is common for two different phases to coexist in the same membrane.

Liquid Phase Hydrocarbon Chain-Melting

One common method of tracking phase transition of lipid membranes is to track hydrocarbon chain-melting through the aforementioned methylene group rocking at 2850cm^{-1} . This measures the conformation freedom of the hydrocarbon chain and can act as a signal of lipid transition between liquid and gel phase(Casal, H. L. and Mantsch, H. H., 1984)[10],(Senak, L., Davies, M. A., and Mendelsohn, R. 1991)[11].

Crystalline and Gel Phase Transition

When doing studies of crystalline and gel phase transitions spectroscopic markers that are sensitive to intermolecular close-contact interactions can be utilized. The sharper peaks caused by the reduction in vibrational energy allows for resolution of interfacial hydration patterns, headgroup-headgroup interactions, headgroup-solvent interactions and also the same hydrocarbon chain packing signals described previously. Often the method to differentiate between gel phase and liquid-gel phases is to follow two distinct signals and watch as one disappears while the other remains(Mantsch, H. H., Madec, C., Lewis, R. N. A. H., and McElhaney, R. N. 1985)[12]. IR spectroscopy can be used to distinguish between crystalline to liquid-crystalline phase transition, but this information requires the signals from several IR markers. (Ruthven N. A. H. Lewis and Ronald N. McElhaney, 2007)[13]. Studies of lipid phase transition has allowed for a deeper understanding of membrane dynamics in cells lending possible explanations of lipid organization in living cells.

Practical Considerations

One major consideration when doing IR spectroscopy on hydrated lipids is avoiding the bands produced from compounds in the buffer solution. The most common solution to this problem is to use a blank to subtract out these bands, but IR radiation may not reach the detector if the absorption bands are very strong. One potential solution to this issue is ATR-FTIR. For this technique the sample is put on in inorganic crystal and the IR radiation is passed through the sample at an angle that is higher than the critical angle of total internal reflection(Frengeli, U. P. 1977)[14]. One potential problem with ATR-FTIR on hydrated lipids is that the materials used can be highly polar which can interact with the samples changing the bilayer's properties(Cevc, G. C. and Marsh, D. 1987)[15]. One benefit of FTIR is the ability to analyze living cells. One application of this method is used to rapidly identify specific species of bacteria. This takes advantage of the fact that different species of bacteria have unique macromolecular makeups. These compositional differences act as fingerprints for microbial identification (Duygu et al., 2009)[16]

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