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Curve Fitting in Raman and IR Spectroscopy: Basic Theory of Line Shapes and Applications

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Key Words

- Collision-induced Scattering
- OMNIC Peak Resolve Software
- Peak Fitting
- Protein Structures
- Vibrational Line Shapes

Introduction

The basic theory behind the origin of line shape is presented and applied to curve fitting. This understanding should enable a user to apply with precision the Peak Resolve routine of OMNIC™. The affect of the line shape parameters on the curve fitting is discussed and a set of literature references is provided for more intense investigation.

The scope of this technical note is limited to the basic theory behind vibrational (IR and Raman) line shapes and how this applies to curve fitting. There are many phenomena, like Fermi Resonance and collision-induced scattering, which affect vibrational line shapes; the reader is directed to the references cited for more information.

Basic Concepts: Origin of Gaussian and Lorentzian Line Shapes

Quantum theory states that molecules possess well defined energy levels. Transitions between these levels, caused when molecules absorb or emit energy, therefore occurs in well defined intervals (quanta), not continuously, which gives rise to the vibrational spectrum. Thus, the absorption of energy by an isolated molecule going from a ground state to a first excited state occurs at a single, well defined, frequency. However, most vibrating molecules exist in a bath of surrounding molecules (the environment), with which they interact. Each molecule interacts with its environment in a slightly different (and dynamic) way, and thus vibrates at a slightly different frequency. The observed line shape is the sum of these individual molecules absorbing or scattering.

At equilibrium, the population of vibrational states is controlled by the Boltzmann distribution. The majority of molecules in a normal IR or Raman experiment initially are in the ground state. Some of these molecules transition to the excited state when the IR radiation or Raman laser strikes the sample. The resulting absorption (IR) or change in scattering (Raman) represents the signal seen by the instrument.

Excited state molecules rapidly return to the ground state – for vibrations, this occurs after a few picoseconds (10^{-12} sec). This relaxation is called the *lifetime* (or *amplitude correlation time*) τ_a. Initially, all of the excited molecules are vibrating together (coherently), but motion and slight differences in vibrational frequencies randomizes this over time. By analogy, coherence in a singing choir produces music, but incoherence produces chaos. The spectrometer can only "see" the molecules while they are both excited and coherently vibrating (singing). As the coherence fades (with *coherence lifetime* τ_c), the now random components interfere, and effectively cancel one another (called dephasing). Although the vibrational energy is not actually lost (the choir is still making noise), the spectrometer can not "see" it – the sum of incoherence is zero.

The effective lifetime τ is a combination of these two components, the coherence lifetime τ_c and the amplitude correlation time τ_a. There are two interesting limiting cases, when $\tau_c >> \tau_a$ or $\tau_c << \tau_a$. Recall again two basics: the overall line shape originates from the sum of all the individual vibrations, and the exact vibrational frequency of a particular molecule is controlled by its environment.

In the first case, where $\tau_c >> \tau_a$, the excited molecule relaxes before incoherence becomes severe. This is the case for solids, because the environment is not in motion. The various molecules of the solid experience a statistical distribution of environments, and the line shape takes on the bell curve or *Gaussian* profile. This profile has the well-known shape from statistics, with a curving (not sharp) center and wings that fall away relatively quickly.

In the second case, where $\tau_c << \tau_a$, the incoherence sets in rapidly, so dephasing is the dominant energy loss channel. This occurs in gases where rotation and collisions happen quickly. The resulting line shape is *Lorentzian* (due to the exponential vibrational population relaxation), which is sharp in the center, but has long wings.

Liquids exist in between these limits, where interactions prevent extremely rapid motion, but the molecules are not locked in place. As a result, the two lifetimes can be close, and the line shape has features of both Gaussian and Lorentzian character. The simplest model for this involves the combination *Gaussian-Lorentzian* (G-L) profile, represented as A*G + (1-A)*L with A (a variable parameter in the fit) being the fraction of Gaussian character ($0 \le A \le 1$). A more complex combination of Gaussian and Lorentzian lines is the *Voigt* profile, where the two characters are convoluted (combined in the Fourier Transform integral). The functional form for a Voigt profile allows the Gaussian and Lorentzian portions to have different line widths.

Each of these profiles is characterized by three parameters: location (frequency), height (intensity) and line width. The Voigt and mixed G-L profiles also include a fourth parameter specifying the relative Lorentzian character.

The location of a peak (x_0) is controlled first by the natural vibrational frequency of the isolated molecule. This leads to the well-known diagrams relating peak locations to functionality. However, the actual peak location also depends upon interactions with the environment. If the molecule is hydrogen bound to neighbors, this effectively lowers the bond energy (spreads the bonding energy over more space), and the peak will red-shift (to lower energy).

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If the molecule is experiencing repulsions, the peak blue shifts (to higher energy). In proteins, this phenomenon leads to the assignment of slightly variant frequencies of the amide I band to different secondary and tertiary structural elements (helices and sheets). Dilution of acetone with either water or carbon tetrachloride shifts the carbonyl band red or blue, respectively.

The height of a peak (Y_0) depends upon the number of molecules present (concentration) and the strength of the absorption (absorptivity). The Beer-Lambert Law uses this for concentration determination. Care in the calibration is needed, however, as dilution can shift the peak as noted already, or there may be peak broadening. The area of the peak is a better indicator of concentration, because, as noted at the outset, the final peak profile is the sum of all the individual elements. In some cases, the peak height can be changed by a broadening mechanism, but the area will remain unchanged as the total number of molecules is constant. Peak height has been used traditionally as being easier to measure; peak fitting allows area to be used, which can improve linearity of calibrations.

Line widths are normally reported as full width at half height (FWHH, Δx). The line width is the most neglected parameter in spectroscopy, and yet it is the richest in information content. All of the dynamics (motion and energy loss) affect the line width, and there are many theories explaining the influence of various environments on line width. At a simple level, the line width is inversely proportional to the effective lifetime τ. Rapid loss of the excitation (short τ) results in broad peaks; a long lifetime (long τ) leads to narrow peaks. This explains the enormously broad O-H peaks of water, as the tight hydrogen bonding network allows the molecules to relax extremely rapidly. Collisions between molecules can also enhance energy loss rates, and may broaden peaks. Molecules in low pressure gases are essentially isolated, so they have no environment to assist in energy loss, resulting in a long lifetime and narrow peaks. See the literature cited on page 4 for a more detailed discussion of line width theory.

A Few Subtleties

There are many phenomena occurring when molecules vibrate which can affect the band shapes. Two common perturbations in IR and Raman are hot bands and isotope effects. Overtones and combination bands can also occur, and other relaxation pathways exist (resonance transfer, Fermi resonance, etc.), all of which alter peak locations and widths – see the literature for more information.

Hot bands result when the first vibrational energy level is significantly populated. Then, the transition from v=1 to v=2 can occur. In a perfect harmonic oscillator, this would occur at the exact same frequency as the v=0 to v=1 transition. However, the phenomenon of anharmonicity lowers the v=2 energy slightly, making the $1 \rightarrow 2$ transition slightly lower in energy than the $0 \rightarrow 1$ transition. If a hot band is present, the vibrational band will exhibit a slight shoulder or hump on the low frequency side. This effect is more pronounced at low frequencies (easier to populate the v=1 level) and in elevated temperature situations.

Isotope effects occur because of the dependence of the harmonic oscillator frequency on the inverse square root of the reduced mass. The reduced mass is essentially the relative mass of the two sides of the oscillator – in a diatomic, the two atoms; in a polyatomic, the mass of the two sides of the bond. As the mass goes up, the frequency goes down. The Raman spectrum of carbon tetrachloride shows five bands at high resolution, due to $^{12}C^{35}Cl_4$, $^{12}C^{35}Cl_3{}^{37}Cl$, $^{12}C^{35}Cl_2{}^{37}Cl_2$, $^{12}C^{35}Cl^{37}Cl_3$ and $^{12}C^{37}Cl_4$, shifting in that order to lower frequencies, and with peak heights proportional to the relative isotopic concentrations of each. These peaks are closely spaced, as the change in reduced mass is small; at low resolution, this makes the peak look distorted and broad. A larger effect occurs in the change from HCl to DCl, which moves the vibration by a factor of $1/\sqrt{2}$, or from roughly 3000 to 2000 cm^{-1}; the peaks are well separated and can be treated independently. The isotope effect has been used in protein analyses, where deuterium exchange shifts some residues, but leaves protected residues unperturbed.

Profile Comparisons

Figure 1 shows the difference between a Gaussian, a Lorentzian and a Voigt profile using the same values for Y_0, x_0 and Δx. The sharper maximum and long wings of the Lorentzian are clearly seen. Mathematical forms for the various line shapes are given in reference 3.

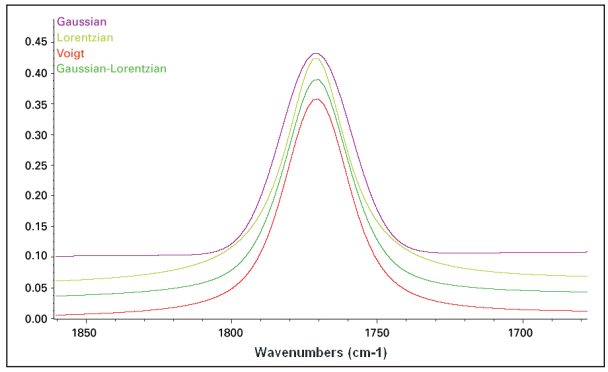

Figure 1: Comparison of general lineshapes: Gaussian, Lorentzian, Voigt and G-L mixed with same parameters

Summarizing from above: the Gaussian profile works well for solid samples, powders, gels or resins. The Lorentzian profile works best for gases, but can also fit liquids in many cases. The best functions for liquids are the combined G-L function or the Voigt profile. The Voigt profile is similar to the G-L, except that the line width Δx of the Gaussian and Lorentzian parts are allowed to vary independently. Figure 2 shows the influence of altering the percent Lorentzian character of a G-L profile, leaving everything else unchanged. The wings grow with A, and the line narrows near the top.

The treatment thus far applies equally to IR or Raman peaks. However, Raman spectra may also contain signals due to fluorescence. Fluorescence involves an electronic transition combined with a vibrational transition. The higher energy needed to boost electrons (visible or UV radiation) explains why this is rare in the IR, but more pronounced with high frequency lasers (depending upon

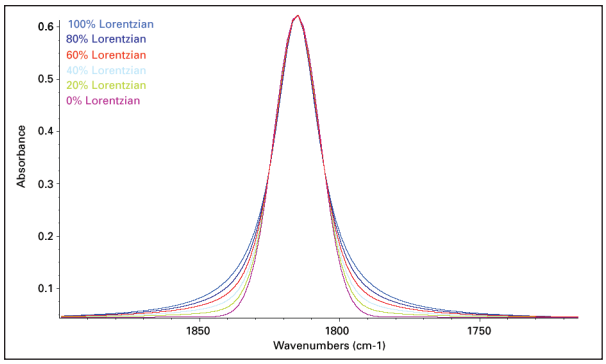

Figure 2: Effect of varying the percent Lorentzian character in a G-L peak. The main influence is visible in the wings.

the sample). Removal of the fluorescence signal can sometimes be accomplished using background removal routines, but curve fitting can also be used, especially when the fluorescence is relatively narrow. Fluorescence tends to exhibit considerable asymmetry, so the *Log-Normal* profile is most often used. The influence of asymmetry on the profile is shown in Figure 3. The log-normal peak can sometimes find applications in analyzing IR and Raman peaks, but fluorescence and chromatography are more common applications of this line shape.

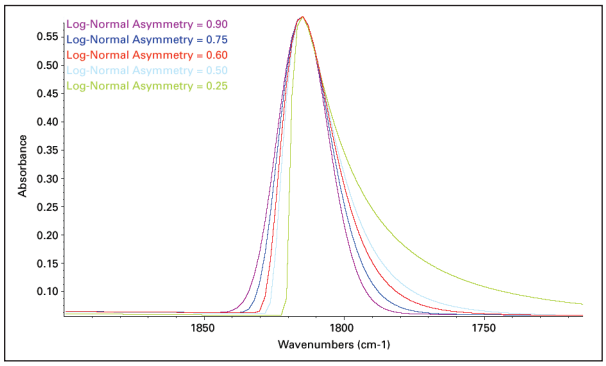

Figure 3: Effect of varying the asymmetry factor in a Log-normal peak. The peak rises more rapidly and has a longer tail as the asymmetry increases.

OMNIC Peak Resolve Operation Notes

The goal of spectral curve fitting is to mathematically create individual peaks from a spectrum that, when added together, match the original data. Convergence is the process used to make this happen. The convergence routine in OMNIC Peak Resolve is a Fletcher-Powell-McCormick algorithm. The convergence point is determined by the ratio of the RMS of the residual of the sum of the created peaks to the RMS noise of the spectrum. Ideally, this should approach 1 as the RMS of the residual approaches the noise. The algorithm converges very rapidly when started with satisfactory parameters.

Curve fitting involves three steps: choice of initial profiles (line shapes and baseline handling), choice of initial parameters (width, height, location), and minimization.

The OMNIC Peak Resolve routine allows the user to select a peak profile for each peak, or to use the same profile for all peaks. The discussion above outlined when certain choices are better – Gaussian for solids, Lorentzian for gases, and mixed (G-L or Voigt) for liquids (and log-normal for fluorescence).

The baseline options allow the user to select none, constant, linear, quadratic or cubic. The fitting routine will use baselines like any other parameter to minimize the residual, sometimes with highly undesirable affects. Thus, selection of the minimum baseline correction is preferable. Choosing "None" works well for previously baseline-corrected data or clean Raman spectra. Baseline correction before fitting is highly recommended if there are peaks on one side of the target peak. Figure 4a shows this for a protein spectrum, where the amide I band is of interest. The choice of linear baseline introduces a severe aberration of the fitting, as the routine minimizes by sloping the baseline into the low frequency region. Selecting none or constant works much better. Use quadratic and cubic baselines with care, as these may actually fit potential peaks.

The user can manually locate initial guesses for peak locations using the peak tool in the lower left corner of the screen, or they can opt for the automated peak locator. Manual selection can be aided by visualizing the second derivative of the spectrum. The minima of the second derivative (upside-down peaks) are probable locations for signals in the original spectrum. The user can locate a peak at each minimum, or can select a larger or smaller number of peaks. The FWHH can be set by typing values into the box or using the Tool Palette region tool; similarly, the noise in a region can be determined by setting a region and choosing Noise.

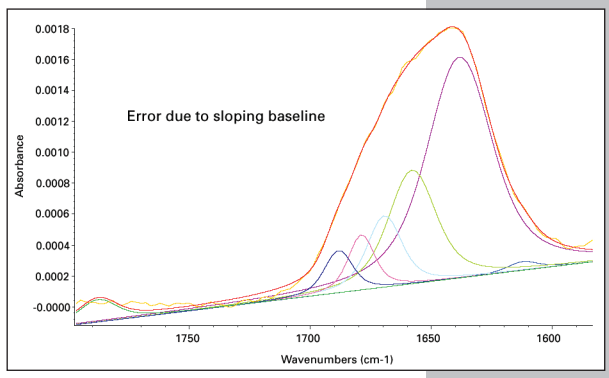

Figure 4a: Effect of a sloping baseline on the quality of a curve fit for a protein. Note the small peaks on the high frequency end, and the fact that the base line passes through the spectrum.

The automated routine uses the defaults selected by the user (shape, width and sensitivity) to set a single peak at the location of each inverted peak of the second derivative. The initial height is set equal to the value of the original spectrum at that point.

In either case, the user can edit the peak table through the "Peaks…" command. Profile shapes, locations, heights, FWHHs, and other parameters (i.e., percent Lorentzian) can be manually altered. Peaks can also be added or deleted either before or after iterations of the fitting routine. Once the edit is complete, the displayed peaks and other information are automatically updated to reflect these changes.

As a general rule, fitting routines converge more effectively the better the initial parameters. For instance, if the initial line width is set to 10% of the actual line width, the minimization routine will sometimes find that peak height drops the residual faster than the peak width. If the peak location is too far from the center of a peak, bad divergence (especially with sloping baselines) can result. As noted, the baseline choice can affect the quality of the fit drastically. Further, the final fit values will "stay home" better if the

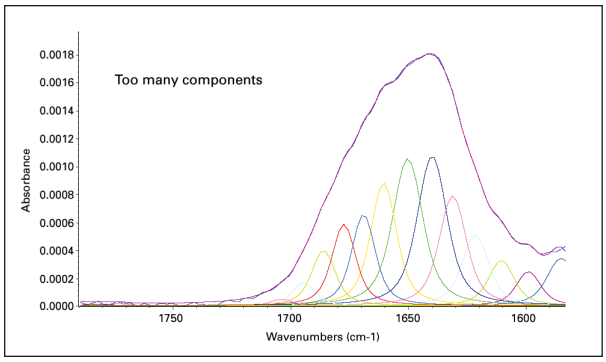

Figure 4b: Effect of using too many components for the same protein peak. The residual overlaps the original spectrum very well, but most of the peaks are scientifically meaningless.

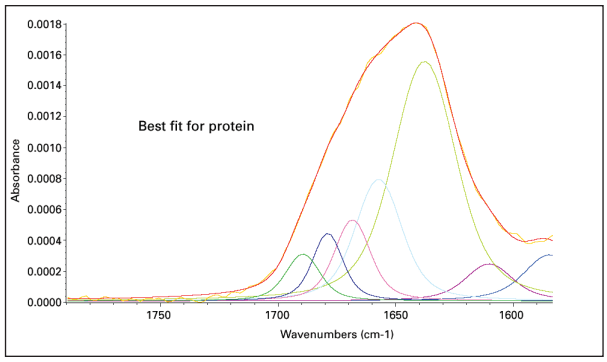

Figure 4c: A good fit to the protein spectrum. The baseline selection was "None" and the number of peak's set to the number suggested by the structure. The ratio of peak areas can now be used to assess the percent of sheet and coil in the protein.

initial estimates are close. Thus, time spent initially will optimize the results.

There are three further considerations in making a curve fit scientifically meaningful. First, a fitting algorithm, given enough peaks and varying parameters, can fit any spectrum. A certain amount of scientific insight is needed to make the peak fitting procedure meaningful. A large number of peaks may give a good visual residual, but be totally meaningless to interpretation – some of the peaks may have no source in reality. Figures 4b and 4c show this for the protein peak – the fit in 4b is excellent, but meaningless. The fit in 4c is the most correct, and can be interpreted in terms of the protein structure. Thus, always start with a smaller number of peaks and increase the number as the user identifies regions in the residual that appear to be the locations of peaks. This iterative procedure can be tedious for broad peaks with multiple humps, while a series of isolated peaks will fit easily the first time through.

Next, the whole point of a fitting routine is to converge to a local minimum of the residual. If there are 10 Lorentzian peaks plus a baseline, the routine is minimizing 32 variables (location, height, width for each plus slope and intercept). Convergence of all these can be tricky in the best of cases. Occasionally, fit routines can "move" peaks a substantial distance to a place in the spectrum where they make no physical sense, but where the convergence is slightly better. Note the small peaks on the high frequency side of Figure 4a – these were used by the fit routine to make the sloping baseline work, but they are obviously meaningless. This is especially a problem if a number of broad peaks are closely spaced. The investigator has the ability in OMNIC Peak Resolve to lock a parameter, which can keep peaks from drifting away from their original location. As more experience is gained using the fitting program, it becomes clear how this works.

Finally, a factor which is of critical importance in fitting is the signal to noise of spectral data, which must be relatively high. The fitting routine will work with some noisy spectra, but noise spikes and low signal contribute to local convergences not related to the signal (the routine tries to fit the noise spikes). The second derivative also becomes unreliable for finding peaks (the derivative of noise is worse noise). Smoothing of data can be used but the problem with smoothing techniques is that they can alter the line shape to some degree. For example, the use of a "heavy" 25-point Savitsky-Golay smooth can completely eliminate peaks. "Gentle" smoothing (5 to 9-point smoothing) can be helpful and should not distort the peak location or peak height of reasonably broad (10 cm^{-1} or more) peaks. However, the line width becomes convoluted with the smoothing function, and must be reported as such. Thus, for best results, efforts should be made experimentally to improve the signal to noise prior to submitting spectra for curve fitting.

Conclusion

Curve fitting opens great power to the end user. The interpretation of protein peaks relies upon curve fitting to extract information about protein structure. The tremendous field of liquid dynamics is accessible, and calibrations based on peak areas, rather than just heights, can be obtained. The fitting routine in OMNIC Peak Resolve was designed to be easy to use, rapidly convergent, and flexible. It provides an additional tool with excellent utility in many fields.

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