Electronic Supplementary Material (ESI) for Analyst.

Supplementary Material for

Recovery of absorbance spectra of micrometer-sized biological and inanimate particles

R. Lukacs*[1], R. Blümel[2] B. Zimmerman[1], M. Bağcıoğlu[1], and A. Kohler[1]

[1]Department of Mathematical Sciences and Technology, Faculty of Environmental Science and Technology, Norwegian University of Life Sciences, 1432 Ås, Norway

[2]Department of Physics, Wesleyan University, Middletown, Connecticut 06459-0155, USA

*Corresponding author: rozalia.lukacs@nmbu.no

S1. Lorentz Model

The Lorentz model is a classical model for the computation of the dielectric constant of a material in the presence of absorption resonances. Although based on classical electrodynamics, including quantum effects only phenomenologically via the (measured) spectrum of discrete molecular frequencies, it is surprisingly effective in explaining the frequency dependence of the complex index of refraction m. In particular, it correctly predicts the Lorentz-type shape of the imaginary part of m, which closely resembles the line shape of an absorption resonance. For the derivation of the Lorentz model, we follow the excellent presentations by Griffiths [1] and Parson [2]. The Lorentz model in its simplest form assumes that an electron is bound to an atom or molecule with a harmonic binding force

$$F_b = -M\omega_0^2 x \quad \text{(S1)}$$

where M is the mass of the electron, ω_0 is the natural oscillation frequency of the electron in the harmonic binding potential and x is the amplitude of vibration of the electron in the direction of the external electric field $\vec{E}$ which, in our case, is the infrared light field. It is not necessary to restrict ourselves to electrons. Any charged particle or active group of charged particles that may execute a vibration, such as, e.g., O-H or C=O stretches, are successfully described by the Lorentz model. Therefore, from now on, we imagine a "particle" with effective mass M and charge q subject to the binding force in Eq. S1, and substitute "electron", or "chemically active group" for "particle", as the case may be. For electrons, for instance, $q = -e$, where $e = 1.602 \times 10^{-19}\ C$ is the elementary charge. For chemically active groups, q is substituted with the polarization charge δq of the polar ends of the group. When the particle vibrates, it loses energy, for instance by electromagnetic dipole radiation or by energy transfer to the backbone molecule or the medium, via the long-range Coulomb force. We model this energy loss with a damping force

$$F_\gamma = -M\gamma\frac{dx}{dt} \quad \text{(S2)}$$

which depends linearly on the speed $\frac{dx}{dt}$ of the vibrating particle, and γ is the damping constant, which depends on the details of the energy dissipation processes. In addition to the binding force F_b and the damping force F_γ, the particle also experiences the driving force

$$F_d = qE(t) + \frac{qP(t)}{3\varepsilon_0} \quad \text{(S3)}$$

where

$$E(t) = E_0 \cos \omega t \quad \text{(S4)}$$

is the field strength of the infrared light as a function of time , ω is its frequency and

$$P(t) = Nqx(t) \quad \text{(S5)}$$

is the polarization induced by the external field and *N* is the number of particles per unit volume. We assume here a linear dielectric, in which the polarization of the medium is directly proportional to the radiation field. Newton's equation of motion for the particle,

$$M\frac{d^2x}{dt^2} = F_b + F_\gamma + F_d \qquad \text{(S6)}$$

leads to the differential equation

$$M\frac{d^2x}{dt^2} + M\gamma\frac{dx}{dt} + M\omega_0^2 x = qE_0\cos\omega t + \frac{qP(t)}{3\varepsilon_0} \qquad \text{(S7)}$$

for the position of the particle. The solution of Eq. S7 is greatly simplified if we consider Eq. S7 as the real part of the complex equation

$$\frac{d^2\tilde{x}}{dt^2} + \gamma\frac{d\tilde{x}}{dt} + \omega_0^2\tilde{x} = \frac{q}{M}\tilde{E} + \frac{q}{3\varepsilon_0 M}\tilde{P} \qquad \text{(S8)}$$

where *x* is the real part of the complex quantity $\tilde{x}$,

$$\tilde{E} = E_0 e^{-i\omega t} \qquad \text{(S9)}$$

is the complex electric field and

$$\tilde{P} = Nq\tilde{x} \qquad \text{(S10)}$$

is the complex polarization. In infrared spectroscopy we are not interested in the transient solutions of Eq. S8, i.e. solutions which are generated by switch-on and switch-off of the infrared light. These solutions quickly decay exponentially in time. Once the transient solutions have decayed, the system settles into the steady-state solution

$$\tilde{x} = \tilde{x}_0 e^{-i\omega t} \qquad \text{(S11)}$$

Inserting this into Eq. S8 and using Eq. S10, we obtain

$$\tilde{x}_0 = \frac{q/M}{\omega_0^2 - \omega^2 - \dfrac{q^2 N}{3\varepsilon_0 M} - i\gamma\omega} E_0 \qquad \text{(S12)}$$

The complex dipole moment of the particle is

$$\tilde{p} = q\tilde{x} = \frac{q/M}{\omega_0^2 - \omega^2 - \dfrac{q^2 N}{3\varepsilon_0 M} - i\gamma\omega} \tilde{E} \qquad \text{(S13)}$$

We now assume that we have N_m active molecules in our sample and each molecule consists of f_s particles with masses M_s, charges q_s, resonance frequencies ω_s and damping constants γ_s. We define

$$\Omega_s^2 = (\omega_s)^2 - \left(\frac{q_s^2 N_m}{3\varepsilon_0 M_s}\right) f_s \qquad \text{(S14)}$$

The polarization $\tilde{P}$, i.e. the dipole moment per unit volume, is given by

$$\tilde{P} = \varepsilon_0 \tilde{\chi} \tilde{E} \qquad \text{(S15)}$$

where ε_0 is the permittivity of the vacuum,

$$\tilde{\chi} = N_m \sum_s \frac{q_s^2 f_s / M_s}{\Omega_s^2 - \omega^2 - i\gamma_s \omega} \qquad \text{(S16)}$$

is the susceptibility and $\tilde{E}$ is the complex electric field defined in Eq. S9. The dielectric constant is

$$\tilde{\varepsilon} = \varepsilon_0 \tilde{\varepsilon}_r \qquad \text{(S17)}$$

where the relative dielectric constant $\tilde{\varepsilon}_r$ depends on $\tilde{\chi}$ according to

$$\tilde{\varepsilon}_r = 1 + \tilde{\chi} \qquad \text{(S18)}$$

In Eq. S16 we have to sum over all resonances over the entire electromagnetic spectrum including the radio frequency region below the infrared frequency range, the infrared frequency region, and the spectral region above the infrared. We are interested in the infrared frequency region. Therefore, for frequencies larger than infrared frequencies, e.g. in the visible and UV, we may neglect $\gamma_s\omega$ with respect to ω^2 and Ω_s^2, and expand Eq. S16 to first order in $\left(\frac{\omega}{\Omega_s}\right)^2$. As a consequence, the summation in this frequency range contributes approximately a real term

$$\alpha(\omega) = N_m \sum_k \frac{q_k^2 f_k / M_k}{\Omega_k^2}\left[1 + \left(\frac{\omega}{\Omega_k}\right)^2\right] \quad \text{(S19)}$$

to the susceptibility in Eq. S16, where the sum over *k* in Eq. S19 extends over all resonances with frequencies above the infrared range. In the case of the resonances below the infrared frequency range, i.e. the far infrared region and radio frequency region, we may neglect Ω_s^2 and $\gamma_s\omega$ with respect to ω^2. Thus, this frequency range, approximately, contributes the frequency dependent term

$$\beta(\omega) = -\frac{N_m}{\omega^2}\sum_l \frac{q_l^2 f_l}{M_l} \quad \text{(S20)}$$

to the susceptibility in Eq. S16, where the sum over *l* in Eq. S20 is over all the resonances below the infrared frequency range. Thus, all together, we now obtain

$$\tilde{\varepsilon}_r = \bar{\varepsilon}_r + N_m \sum_{s \,\epsilon\, IR} \frac{q_s^2 f_s / M_s}{\Omega_s^2 - \omega^2 - i\gamma_s\omega} \quad \text{(S21)}$$

where the sum in Eq. S21 extends only over the infrared (IR) resonances and

$$\bar{\varepsilon}_r = 1 + \alpha(\omega) + \beta(\omega) \quad \text{(S22)}$$

is the frequency dependent effective relative dielectric constant of the medium, i.e. the background dielectric constant, on which the infrared resonances are built. The complex index of refraction is now given by

$$m = \sqrt{\tilde{\varepsilon}_r} \qquad \text{(S23)}$$

At this point two important comments are in order: (1) In optics we are familiar with the phenomenon of dispersion, i.e. the change of the index of refraction with increasing wavelength. For glass, for instance, we know that the index of refraction decreases with increasing wavelength, which gives rise to the familiar observation of the splitting of white light into its constituent colours with the help of a prism. This decrease in the index of refraction is now easily explained. According to Eqs. S19 and S20, both $\alpha(\omega)$ and $\beta(\omega)$ cause a decrease in $\bar{\varepsilon}_r$, and therefore, according to Eq. S23, they also cause a decrease in *m*, when the wavelength increases. Therefore the Lorentz model explains this basic observation. (2) Since the quantities N_m, q_s , f_s, and γ_s are usually not readily available, we write

$$\tilde{\varepsilon}_r = \bar{\varepsilon}_r + \sum_{s \in IR} \frac{\Lambda_s}{\tilde{V}_s^2 - \tilde{\nu}^2 - i\Gamma_s\tilde{\nu}} \qquad \text{(S24)}$$

where $\bar{\varepsilon}_r$, $\tilde{V}_s$, Λ_s, and Γ_s are adjustable parameters and

$$\tilde{\nu} = \frac{1}{\lambda} = \frac{\omega}{2\pi c} \qquad \text{(S25)}$$

where λ is the wavelength and *c* is the vacuum speed of light. This is the microscopic basis for the usual practice in spectroscopy of fitting Lorentzian lines to resonance structures in the index of refraction.

Instead of using quantum mechanics to solve for the quantized excitations of the molecule in the presence of the infrared radiation field, the Lorentz model uses classical mechanics to solve the forced, damped harmonic oscillator equation (S8). This, apparently, introduces two errors, (i) the oscillator (S8) is not quantized and (ii) neither is the radiation field, i.e. it is not treated as consisting of photons. The question is: how serious are these approximations? The answer is the following. (i) Quantum mechanics has been partially included by providing the Lorentz model with the discrete set of molecular frequencies Ω_s, a direct result of the quantization of the molecule via the many-body Schrödinger equation. (ii) Although the radiation field consists of photons, the light intensities in infrared spectroscopy are so high that we can safely neglect the quantization of the radiation field. Of course there remains the question of the quantization of the Ω_s modes, whose amplitudes are treated as a classical, continuous variable, although, according to quantum mechanics, they should be quantized. This, however, is not serious. As soon as the Ω_s oscillators are appreciably excited, corresponding to a few absorbed photons, the classical approximation is practically indistinguishable from the exact quantum treatment, which is due to the Bosonic nature of the oscillator excitations. Thus, because of the relatively large intensities of the infrared light field, the classical approximation of both the molecular oscillators and the radiation field is justified.

S.2. Mie Formulas

The Mie scattering amplitudes are defined as:

$$S_1(\theta) = \sum_{n=1}^{\infty} \frac{2n+1}{n(n+1)} \{a_n \pi_n(cos\theta) + b_n \tau_n(cos\theta)\} \qquad \text{(S26)}$$

$$S_2(\theta) = \sum_{n=1}^{\infty} \frac{2n+1}{n(n+1)} \{b_n \pi_n(cos\theta) + a_n \tau_n(cos\theta)\} \qquad \text{(S27)}$$

$$a_n = \frac{\psi_n^{'}(y)\psi_n(x) - m\psi_n(y)\psi_n^{'}(x)}{\psi_n^{'}(y)\zeta_n(x) - m\psi_n(y)\zeta_n^{'}(x)} \qquad \text{(S28)}$$

$$b_n = \frac{m\psi_n^{'}(y)\psi_n(x) - \psi_n(y)\psi_n^{'}(x)}{m\psi_n^{'}(y)\zeta_n(x) - \psi_n(y)\zeta_n^{'}(x)} \quad \text{(S29)}$$

$$\psi_n(z) = \sqrt{\frac{\pi z}{2}} J_{n+\frac{1}{2}}(z) \quad \text{(S30)}$$

$$\zeta_n(z) = \sqrt{\frac{\pi z}{2} H^{(2)}_{n+\frac{1}{2}}(z)} \quad \text{(S31)}$$

$$x = 2\pi a\tilde{\nu} \quad \text{(S32)}$$

$$y = mx \quad \text{(S33)}$$

$$m = n + in' \quad \text{(S34)}$$

$$\pi_n(cos\theta) = \frac{1}{sin\theta} P^1_n(cos\theta) \quad \text{(S35)}$$

$$\tau_n = \frac{d}{d\theta} P^1_n(cos\theta) \quad \text{(S36)}$$

where, *m* (complex in general) is the refractive index of the homogenous sphere, *a* is the radius of the sphere, *J* is the Bessel function of the 1st kind and $H^{(2)}$ denotes the Hankel functions. The argument *z* in Eqs. S30 and S31 is an arbitrary complex number; it may be equal to x or y. P^1 denotes the first order associated Legendre polynomial.

The purpose of listing these equations is twofold: It establishes our notation and shows that all aspects of Mie scattering may indeed be written down analytically. In this context we mention that *m* in this paper is defined according to S34, with negative *n'* for positive absorbance. This is the opposite sign convention from the one used in the standard reference book of Van De Hulst [3].

S.3. Iterative algorithm

Initialization: For $j = 1$, the complex refractive index $m_j = n_j + i\,n'_j$ is initialized, where j is the index of iteration. The real part of the refractive index, n_j, is initialized with an estimated constant n_0. The imaginary part, n'_j, is initialized with zero.

Following initialization, the iteration proceeds as follows:

I. The formulas in supplementary materials, part S.2., together with Eq. 18 are used to predict the absorbance spectrum $A^{(j)}$ from the complex refractive index m_j.

II. The difference $E^{(j)}$ between the measured spectrum $A_{measured}$ and the predicted spectrum $A^{(j)}$ is calculated according to:

$$E^{(j)}(\tilde{\nu}) = A_{measured}(\tilde{\nu}) - A^{(j)}(\tilde{\nu}) \qquad \text{(S37)}$$

III. From the difference and using Beer-Lambert's law[2], the next value for n' is calculated according to:

$$n'_{j+1}(\tilde{\nu}) = n'_j(\tilde{\nu}) + \frac{ln(10)}{4\pi\tilde{\nu}d_{eff}}E^{(j)}(\tilde{\nu}) \quad , \qquad \text{(S38)}$$

where $d_{eff} = \frac{4a}{3}$ is the effective thickness of a sphere of radius a.

IV. The negative values of $n'(\tilde{\nu})$ are set to zero.

V. A new value for n $(\tilde{\nu})$ is predicted according to:

$$n_{j+1}(\tilde{\nu}) = n_0 + Kramers\ Kronig\left[n'_{j+1}(\tilde{\nu})\right] \qquad \text{(S39)}$$

VI. The new complex refractive index is calculated according to:

$$m_{j+1}(\tilde{\nu}) = n_{j+1}(\tilde{\nu}) + i\,n'_{j+1}(\tilde{\nu}) \qquad \text{(S40)}$$

The complex refractive index $m_{j+1}(\tilde{\nu})$ is updated and the next iteration with $j+1$ is started.

1. D. J. Griffiths, *Introduction to Electrodynamics*, 3 edn., Prentice-Hall, Upper Saddle River, 1999.
2. W. W. Parson, *Modern Optical Spectroscopy*, Student Edition edn., Springer, Heidelberg, 2009.
3. H. C. v. d. Hulst, *Light scattering by small particles*, Wiley, New York,, 1957.