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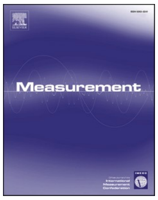

Measurements of optical properties of liquids in a quartz cuvette: Rigorous model, uncertainty analysis and comparison with popular approximations

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ABSTRACT

In this paper we construct and comprehensively analyze the strict optical model of a quartz cuvette filled with investigated liquid, that is typically used in spectrophotometry measurements. We do not make any assumptions concerning the scale of reflections or attenuations but we assume no scattering. We perform uncertainty analysis assuming uncertainties of transmittance and reflectance close to those met in our experiments. Neglection of the reflectance, which is typical for popular approximations, makes the calculation of the real part of the liquid refractive index impossible and introduces systematic errors to the calculations of the absorption coefficient. Therefore, we calculate values of these systematic errors and we compare them to the uncertainties. This allows us to determine the accuracy of popular approximations, particularly, we demonstrate that an approximation in which the transmittance is normalized to the transmittance of a cuvette filled with pure non-absorbing solvent is valid.

1. Introduction

Spectrophotometry is one of the most basic and common experimental techniques in analytical chemistry. The quantity that is directly measured is the transmittance T defined as this part of the intensity of the incident light that passes through an investigated semi-transparent sample. Transmittance can be measured for one particular wavelength λ or as a function of the wavelength in a finite spectral range. Knowledge about $T(\lambda)$ dependence can be used just as it is, e.g. for material identification, or the transmittance can be further transformed into the absorbance A in order to use the Beer-Lambert law [1,2]. A popular form of this law states that the light absorbance of a solution, taken as the decimal logarithm of transmittance, is proportional to the molar attenuation coefficient ε and the concentration of absorbing species c that are diluted in a non-attenuating solvent, and to the optical path d. Formally:

$$A = -\log_{10}T = \varepsilon \cdot c \cdot d. \tag{1}$$

The Beer-Lambert law allows for convenient (using the purely optical method) determination of the value of the concentration of a chemical species in a solution when its attenuation coefficient is known, or for calculation of the attenuation coefficient of a chemical species diluted in a solvent when its concentration is known. Limitations of the Beer-Lambert law are nicely discussed in [3], whereas [4] underlines the problem of the shortcomings of the very simple optical model that is silently assumed in Eq. (1). Briefly, Eq. (1) neglects all reflections in the optical system as well as the existence of the cuvette surrounding the investigated liquid. Because it is not possible to precisely calculate the attenuation coefficient knowing only the transmittance, some systematic errors are introduced into the calculations. To the best of our knowledge the most advanced analysis of the optical model of a liquid in a cuvette, however still not strict, was done by Soares, who also proposed “the correction of systematic errors from the reference liquid’s spectral nonflatness as well as for the refractive-index discrepancies between the sample and the reference liquids and for the multiple beam reflections” [5].

In this paper, we present and discuss a rigorous model that describes exactly the typical experimental setup used in spectrophotometry – a cuvette filled with liquid (Fig. 1). We show how to calculate the complex refractive index n of both the cuvette and the liquid from the intensity of the transmitted I_t and reflected I_r light, and we analyze their uncertainties. Next, we calculate what systematical errors are introduced into the calculation of attenuation coefficient using Eq. (1), i.e. when only transmittance is taken into account, for three cases: when the reference measurement is performed for cuvette with a pure solvent (non-absorbing, $c = 0$), when the reference measurement is performed for empty cuvette, and when the reference measurement is performed

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without cuvette and liquid.

2. Methods

2.1. Considered optical setup

Fig. 1 illustrates the considered experimental setup. It is a typical configuration used in spectrophotometry, where the investigated liquid is placed in a quartz cuvette. The cuvette is illuminated from the left side by a beam of typically monochromatic light characterized by the intensity I_i. The incident light passes through four interfaces (air/quartz, quartz/liquid, liquid/quartz, quartz/air) at which it can be reflected and three media (quartz, liquid, quartz) in which it can be attenuated. Values that are measured are intensities of light that is reflected from the whole experimental setup I_r and that that is transmitted through the whole experimental setup I_t. Because the dimensions of the quartz cuvette (~1 mm) are large in comparison to the wavelength of the incident light (UV, VIS, NIR), we assume incoherent interference, which implies that we are working with intensities and not with amplitudes. We do not make any assumptions concerning the scale of reflections or attenuations, however, we assume that the light beam is perpendicular to all interfaces. We also assume that the investigated liquid is homogeneous at least at the scale of the incident light wavelength since we use the formalism of electromagnetism of continuous media. In this work we do not consider scattering, i.e. all the losses are attributed to the absorption. As a consequence attenuation, extinction and absorption are equivalent terms. The problem of separation of absorption and scattering coefficients from the extinction coefficient, including experimental technique, has been recently discussed in [6]. Meaning of all symbols used in Fig. 1 will be introduced consecutively in the text.

2.2. Refractive index

The refractive index n is one of the possibilities to represent the optical properties of matter. Particularly, complex refractive index $n = n_1 + in_2$, where $n_1, n_2 \in \mathbb{R}$, provides a convenient and complete description of optical properties when absorption has to be taken into account. Formally, $\varepsilon_r = \varepsilon/\varepsilon_0 = \varepsilon_r^{'} + i\varepsilon_r^{''=n}2 = (n_1 + in_2)^2 = (n_1^2 - n_2^2) + 2in_1n_2$, where ε is the permittivity, which we note is a complex value. Since the value of the wave vector $k = nk_0 = \frac{2\pi n}{\lambda_0}$, the electric field intensity of an electromagnetic plane wave that travels along x-axis can be written as [7]:

$$\mathbf{E}(x,t) = \mathbf{E}_0 \cdot e^{-i(\omega t - kx)} = \mathbf{E}_0 \cdot e^{-i(\omega t - nk_0 x)} = \mathbf{E}_0 \cdot e^{-i(\omega t - n_1 k_0 x - in_2 k_0 x)} = \mathbf{E}_0 \cdot e^{-i(\omega t - n_1 k_0 x)} e^{-n_2 k_0 x}. \tag{2}$$

Magnetic field intensity has a similar form. Above equation straightforwardly illustrates the fact that the real part of refractive index n_1 affects the phase of the plane wave whereas the imaginary part n_2 determines space attenuation of the electric and magnetic field intensities and is directly related to absorption coefficient defined as:

$$I(x) = I_0 e^{-\alpha \cdot x} \quad I = \frac{1}{2}\mathrm{Re}(\mathbf{E} \times \mathbf{H}^\star) \quad \alpha = 2n_2 k_0 = \frac{4\pi n_2}{\lambda_0}, \tag{3}$$

where asterisk ($\star$) denotes complex conjugation. A medium is considered lossy when $\alpha > 0$ or equivalently $n_2 > 0$.

In our work, we describe the optical properties of all media (medium 1 – air, medium 2 – quartz and medium 3 – liquid) by refractive indices and we treat them as dielectrics. We note that we do not make any assumptions on the scale of the absorption coefficients and thus both the quartz cuvette and investigated liquid are described by complex refractive indices. Low optical losses of quartz cuvette should result from reference measurement and further calculations, not from a priori assumptions. Only air is treated as lossless, however, it is not problematic to make it lossy too.

2.3. Fresnel equations

When an electromagnetic plane wave incidents from medium 1, characterized by refractive index $n_1 = n_{11} + in_{12}$, in direction of medium 2, characterized by refractive index $n_2 = n_{21} + in_{22}$, some part of this wave is reflected and some part is transmitted. The strength of the electric field of the reflected wave equals $E_r = r_{12} \cdot E_I$, the strength of the magnetic field of the reflected wave equals $H_r = -r_{12} \cdot H_I$, the strength of the electric field of the transmitted wave equals $E_t = t_{12} \cdot E_I$, and the strength of the magnetic field of the transmitted wave equals $H_t = t_{12} \cdot H_I$, where r_{12} and t_{12} are Fresnel reflection and transmission coefficients, E_I stands for strength of the electric field of the incident wave, $\frac{E_i}{H_i} = \frac{E_r}{H_r} = \frac{376.7\Omega}{n_1}$, and $\frac{E_t}{H_t} = \frac{376.7\Omega}{n_2}$ (please be aware of redefinition of the SI base units in 2019). The exact equations for the r_{12} and t_{12} coefficients are derived from the boundary conditions for electric and magnetic fields at the interface between two dielectric material and have the following forms:

$$1 + r_{12} = t_{12} \quad n_1 - n_1 r_{12} = n_2 t_{12} \quad r_{12} = \frac{n_1 - n_2}{n_1 + n_2} \quad t_{12} = \frac{2n_1}{n_1 + n_2}. \tag{4}$$

Related intensity coefficients R_{12} and T_{12} that link the time-averaged power density flux I carried by the incident, reflected and transmitted electromagnetic waves are usually defined as follows:

$$R_{12} = \frac{I_{r,12}}{I_{i,12}} = r_{12} \cdot r_{12}^\star \quad T_{12} = \frac{I_{t,12}}{I_{i,12}} = \frac{n_{21}}{n_{11}} \cdot t_{12} \cdot t_{12}^\star. \tag{5}$$

We also note that R stands for reflectance from the whole experimental setup and equals $R = \frac{I_r}{I_i}$, whereas R_{kl} stands for reflectance on particularly interface between medium k and l. Similarly, T stands for transmittance through the whole experimental setup and equals $T = \frac{I_t}{I_i}$, whereas T_{kl} stands for transmittance through particularly interface between medium k and l.

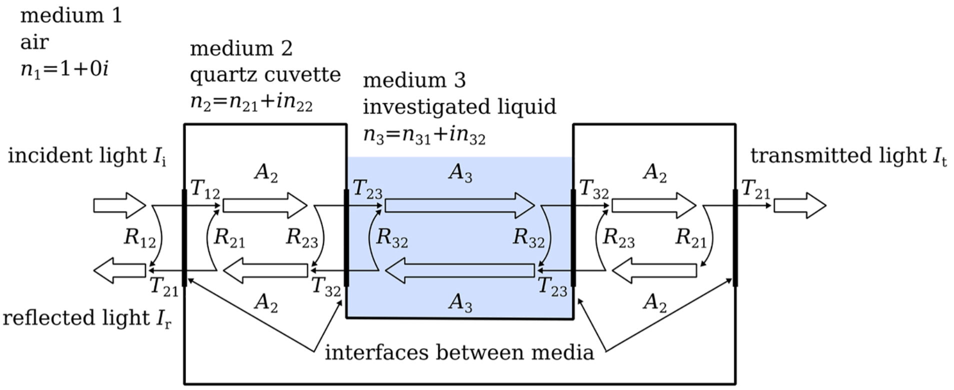

Fig. 1. Schematics of experimental setup illustrating possible reflections and attenuations of the incident light.

3. Rigorous model

3.1. Formalism

To mathematically describe light propagation in the optical setup illustrated in Fig. 1 we use transfer matrix formalism. We assume incoherent interference, which implies that we are working with intensities and not with amplitudes. It is easy to modify this approach to include coherent or partially coherent interference, as described in [8].

Let's consider relations between intensities of the right-going (+) and left-going (−) light on the left (L) and right (R) side of the interface between medium 1 and medium 2 in a typical for transfer-matrix method scheme:

$$\begin{pmatrix} I_{1R}^{+} \\ I_{1R}^{-} \end{pmatrix} = T_{12}^{-1}\cdot\begin{pmatrix} 1 & -R_{21} \\ R_{12} & T_{12}T_{21}-R_{12}R_{21} \end{pmatrix}\cdot\begin{pmatrix} I_{2L}^{+} \\ I_{2L}^{-} \end{pmatrix} \tag{6}$$

Next, let's consider changes in the intensity of light passing through medium 2 from one interface to the second interface:

$$\begin{pmatrix} I_{2L}^{+} \\ I_{2L}^{-} \end{pmatrix} = \begin{pmatrix} A_2^{-1} & 0 \\ 0 & A_2 \end{pmatrix}\cdot\begin{pmatrix} I_{2R}^{+} \\ I_{2R}^{-} \end{pmatrix}, \tag{7}$$

where $A_2 = e^{-\alpha_2\cdot d_2}$ stands for attenuation of the intensity, α_2 for the absorption coefficient of medium 2, and d_2 for the geometrical length (thickness of the cuvette wall). Having that we can write full expression linking the intensity of the incident, reflected and transmitted light:

$$\begin{aligned} \begin{pmatrix} I_i \\ I_r \end{pmatrix} \quad = \quad & T_{12}^{-1}\cdot\begin{pmatrix} 1 & -R_{21} \\ R_{12} & T_{12}T_{21}-R_{12}R_{21} \end{pmatrix}\cdot\begin{pmatrix} A_2^{-1} & 0 \\ 0 & A_2 \end{pmatrix}\cdot T_{23}^{-1}\cdot\begin{pmatrix} 1 & -R_{32} \\ R_{23} & T_{23}T_{32}-R_{23}R_{32} \end{pmatrix}\cdot\begin{pmatrix} A_3^{-1} & 0 \\ 0 & A_3 \end{pmatrix}\cdot \\ & T_{32}^{-1}\cdot\begin{pmatrix} 1 & -R_{23} \\ R_{32} & T_{32}T_{23}-R_{32}R_{23} \end{pmatrix}\cdot\begin{pmatrix} A_2^{-1} & 0 \\ 0 & A_2 \end{pmatrix}\cdot T_{21}^{-1}\cdot\begin{pmatrix} 1 & -R_{12} \\ R_{21} & T_{21}T_{12}-R_{21}R_{12} \end{pmatrix}\cdot\begin{pmatrix} I_t \\ 0 \end{pmatrix} \end{aligned}, \tag{8}$$

where $A_3 = e^{-\alpha_3\cdot d_3}$ stands for attenuation of the intensity, α_3 for the absorption coefficient of medium 3 and d_3 for the geometrical length (distance between walls inside the cuvette). Now we set $I_i = 1$, $I_r = R$, $I_t = T$, R_{kl} and T_{kl} according to Eqs. (4) and (5), and A_2 and A_3 according to Eqs. (7) and (8). This reduces the number of unknown parameters to four: two for quartz cuvette n_{21} and n_{22}, and two for the investigated liquid n_{31} and n_{32}. It is convenient to rewrite Eq. (8) in the following form:

$$\begin{pmatrix} 1 \\ R \end{pmatrix} = \begin{pmatrix} M_{11}(n_{21},n_{22},n_{31},n_{32}) & M_{12}(n_{21},n_{22},n_{31},n_{32}) \\ M_{21}(n_{21},n_{22},n_{31},n_{32}) & M_{22}(n_{21},n_{22},n_{31},n_{32}) \end{pmatrix}\cdot\begin{pmatrix} T \\ 0 \end{pmatrix}, \tag{9}$$

which led to the final formula:

$$\begin{aligned} T &= M_{11}^{-1}(n_{21},n_{22},n_{31},n_{32}) \\ R &= M_{21}(n_{21},n_{22},n_{31},n_{32})\cdot M_{11}^{-1}(n_{21},n_{22},n_{31},n_{32}) \end{aligned}. \tag{10}$$

We note that technically, it is required to make the measurements twice: the first time for empty cuvette, setting $n_{31} = n_{11} = 1$ and $n_{32} = n_{12} = 0$, in order to find of n_{21} and n_{22}; and the second time for filled cuvette in order to find n_{31} and n_{32} using previously found values of n_{21} and n_{22}. Technically it is not problematic since many modern spectrophotometers are double beam (another name dual-beam).

3.2. Example

Let's assume we are using a quartz cuvette manufactured by BIOSENS – model 1QS2 (2), with walls of the thickness $d_2 = 1.25$ mm and distance between walls inside the cuvette that equals $d_3 = 2$ mm, like in our previous work [9]. Next, let's assume that for the wavelength $\lambda = 500$ nm we measured the intensity of reflected and transmitted light for empty cuvette obtaining $R_{\text{empty}} = 0.1138$ and $T_{\text{empty}} = 0.8799$, and the intensity of the reflected and transmitted light for cuvette filled with unknown liquid obtaining $R_{\text{filled}} = 0.0436$ and $T_{\text{filled}} = 0.5630$. Our goal is to find the complex refractive index of the cuvette and the liquid.

First, we have to solve the following system of implicit equations:

$$\begin{aligned} T_{\text{empty}} &= 0.8799 = M_{11}^{-1}(n_{21},n_{22},1,0) \\ R_{\text{empty}} &= 0.1138 = M_{21}(n_{21},n_{22},1,0)\cdot M_{11}^{-1}(n_{21},n_{22},1,0) \end{aligned}. \tag{11}$$

This can be done using one of the many available mathematical software, here we used Wolfram Mathematica v.12. As a results we get $n_{21} = 1.43$, and $n_{22} = 9.58\cdot10^{-8}$. Using Eq. (7) we get $\alpha_2 = 2.4\text{m}^{-1}$.

Next, we have to solve a similar system of equations but this time for filled cuvette:

$$\begin{aligned} T_{\text{filled}} &= 0.5630 = M_{11}^{-1}\left(1.43, 9.58\cdot10^{-8}, n_{31}, n_{32}\right) \\ R_{\text{filled}} &= 0.0436 = M_{21}\left(1.43, 9.58\cdot10^{-8}, n_{31}, n_{32}\right)\cdot M_{11}^{-1}\left(1.43, 9.58\cdot10^{-8}, n_{31}, n_{32}\right) \end{aligned}. \tag{12}$$

As a result we get $n_{31} = 1.33$, and $n_{32} = 1.0\cdot10^{-5}$. Using Eq. (8) we get $\alpha_3 = 251\text{m}^{-1}$.

4. Results

4.1. Empty cuvette

Fig. 2 shows the results of the calculation of transmittance through and reflectance from an empty cuvette (filled with air $n_1 = 1$, geometrical dimensions like in the case of cuvette from Section 3.2) as a function of the real and imaginary part of its refractive index n_2. We are aware that a typical quartz cuvette can be described by the real part of n_2 of the order 1.43, and the imaginary part of the order of 10^{-7} or even lower and the range of considered values is much larger than required, but we want to illustrate trends occurring in this five-layer optical system (air/quartz/air/quartz/air). Moreover, Fig. 2a can be used for direct estimation of n_{21} and n_{22} from R and T for optical system with the same thickness of walls regardless of the value of the optical length of the interior. The main conclusion that can be drawn is that both transmittance and reflectance depend significantly both on the real and imaginary parts of the refractive index. Transmittance is most sensitive to changes of n_{21} when n_{21} is in the range 1.53–1.59 depending on the n_{22} value, and is most sensitive to changes of n_{22} when n_{22} is close to zero. Reflectance is most sensitive to changes of n_{21} when n_{21} is in the range 1.53–1.70 depending on the n_{22} value, and is most sensitive to changes of n_{22} when n_{22} is close to zero. Both transmittance and reflectance start to be insensitive on n_{21} when of n_{21} approaches unity.

4.2. Uncertainty analysis – Derivatives

Despite our considerations being purely theoretical, it is not only worth it but also necessary to analyze the uncertainties of the extracted values of n_{21} and n_{22}, assuming conditions similar to this occurring in real experiments, i.e. that R_{empty} and T_{empty} are measured directly and are disturbed by Gaussian noise with zero mean value and standard devia-

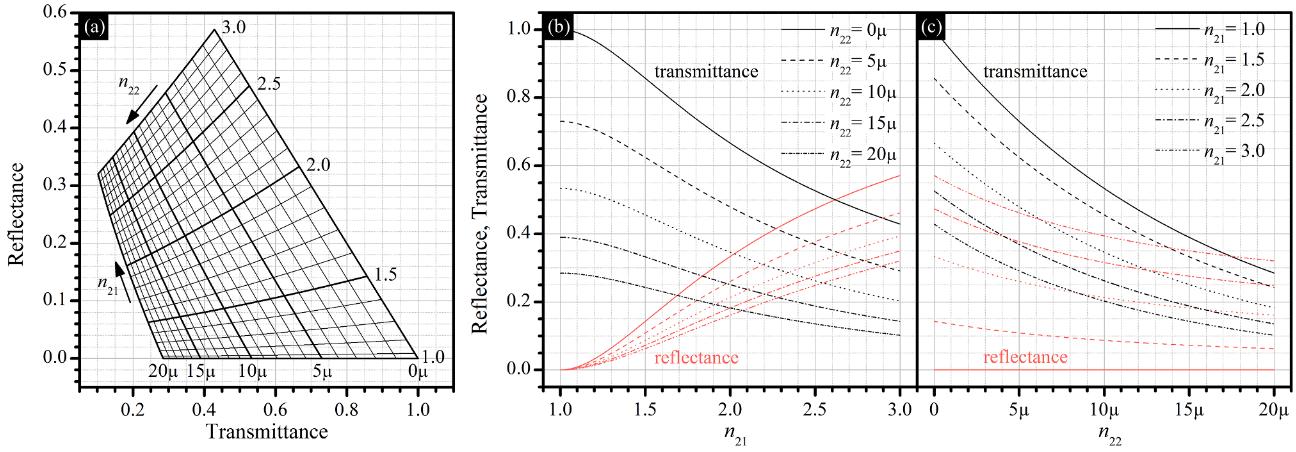

Fig. 2. (a) Dependence of the transmittance and reflectance (T, R) on the real and imaginary parts of the refractive index of a cuvette (n_{21}, n_{22}) in a case when the cuvette is empty. (b) Dependence of the transmittance T or reflectance R on the real part of the refractive index of a cuvette n_{21} for selected values of the imaginary part n_{22}. (c) Dependence of the transmittance T or reflectance R on the imaginary part of the refractive index of a cuvette n_{22} for selected values of the real part n_{21}.

tion value of 0.0025. Thus $u(R_{\text{empty}}) = u(T_{\text{empty}}) = 0.0025$, which well describes our experimental setup [9]. The uncertainty of n_{21} and n_{22} can be expressed as:

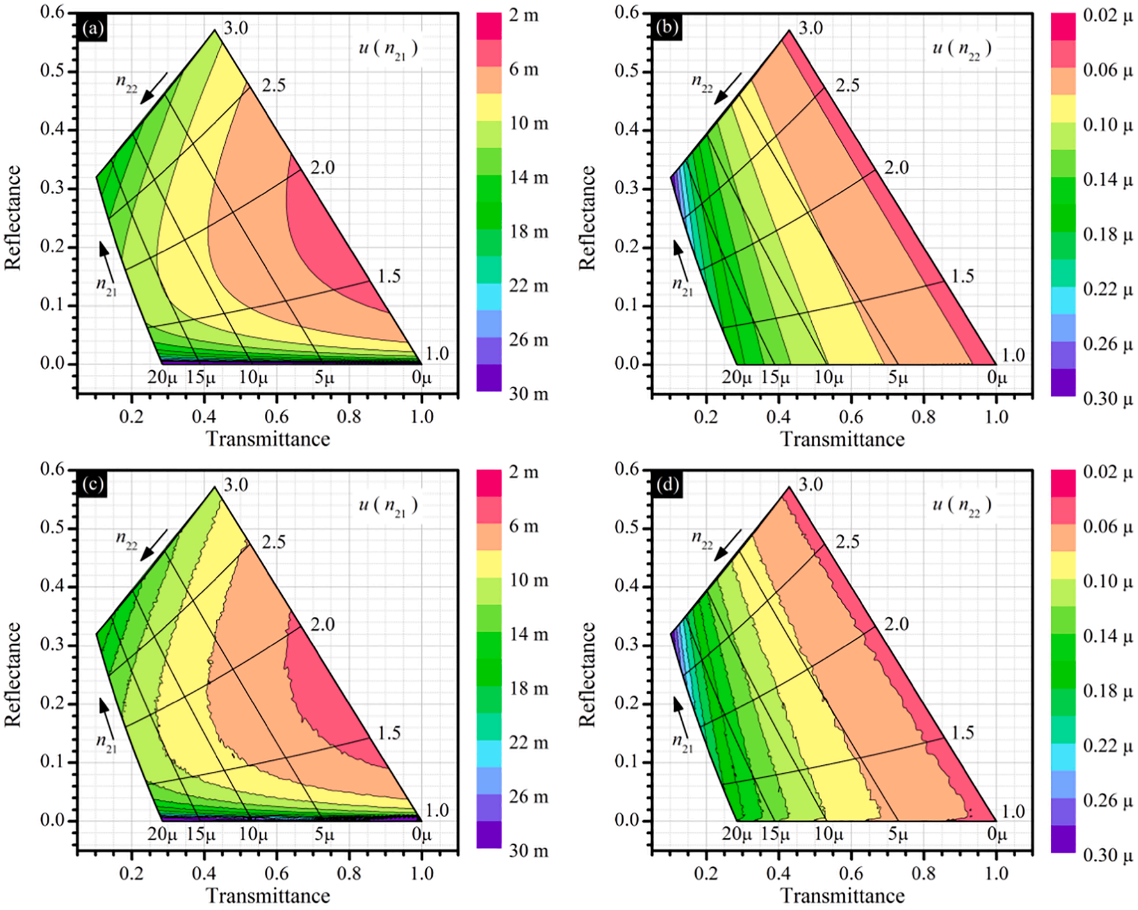

Fig. 3. (a,c) Uncertainty of the real part $u(n_{21})$ of the refractive index of a cuvette and (b,d) uncertainty of the imaginary part $u(n_{22})$ of the refractive index of a cuvette as a function of transmittance and reflectance (T, R) or equivalently as a function of the real and imaginary parts of the refractive index of a cuvette (n_{21}, n_{22}) in a case when the cuvette is empty (filled with air). Results presented in (a) and (b) are obtained using a derivative approach, results presented in (c) and (d) are obtained using Monte Carlo simulations. In all cases $u(T) = u(R) = 0.0025$.

$$u(n_{21}) = \sqrt{\left(\frac{\partial n_{21}}{\partial T_{\text{empty}}}\right)^2 u^2\left(T_{\text{empty}}\right) + \left(\frac{\partial n_{21}}{\partial R_{\text{empty}}}\right)^2 u^2\left(R_{\text{empty}}\right)}$$
$$u(n_{22}) = \sqrt{\left(\frac{\partial n_{22}}{\partial T_{\text{empty}}}\right)^2 u^2\left(T_{\text{empty}}\right) + \left(\frac{\partial n_{22}}{\partial R_{\text{empty}}}\right)^2 u^2\left(R_{\text{empty}}\right)}. \tag{13}$$

The only problem is that derivatives of n_{21} and n_{22} with respect to T_{empty} and R_{empty} cannot be calculated directly since indeed there is an analytical expression for $T_{\text{empty}}(n_{21}, n_{22})$ and for $R_{\text{empty}}(n_{21}, n_{22})$ but there is no analytical expression neither for $n_{21}\left(T_{\text{empty}}, R_{\text{empty}}\right)$ nor for $n_{22}\left(T_{\text{empty}}, R_{\text{empty}}\right)$ (they are implicit functions). A possible solution is to use the inverse function theorem that states that the Jacobian of the inverse function is the inverse Jacobian of the function, namely:

$$J = \frac{\partial\left(T_{\text{empty}}, R_{\text{empty}}\right)}{\partial(n_{21}, n_{22})} = \begin{pmatrix} \frac{\partial T_{\text{empty}}}{\partial n_{21}} & \frac{\partial T_{\text{empty}}}{\partial n_{22}} \\ \frac{\partial R_{\text{empty}}}{\partial n_{21}} & \frac{\partial R_{\text{empty}}}{\partial n_{22}} \end{pmatrix} \quad J^{-1} = \frac{\partial(n_{21}, n_{22})}{\partial\left(T_{\text{empty}}, R_{\text{empty}}\right)} = \begin{pmatrix} \frac{\partial n_{21}}{\partial T_{\text{empty}}} & \frac{\partial n_{21}}{\partial R_{\text{empty}}} \\ \frac{\partial n_{22}}{\partial T_{\text{empty}}} & \frac{\partial n_{22}}{\partial R_{\text{empty}}} \end{pmatrix}. \tag{14}$$

Thus the calculation of the derivatives of the real and imaginary parts of the refractive index with respect to transmittance and reflectance is replaced by the calculation of the derivative of the transmittance and reflectance with respect to real and imaginary parts of the refractive index, proper matrix construction, matrix inversion, and matrix readout, taking into account that for example $\frac{\partial T_{\text{empty}}}{\partial n_{22}}$ and $\frac{\partial n_{22}}{\partial T_{\text{empty}}}$ are in different places of the matrix J and J^{-1}.

The results of uncertainty calculations are shown in Fig. 3a and Fig. 3b. Concluding Fig. 3a one can see that the absolute value of the uncertainty of the real part of the refractive index of the empty cuvette rarely exceeds 0.02, except when n_{21} approaches unity, where dependence of transmittance on n_{21} disappears. Dependence of uncertainty on n_{21} is nonmonotonic and for every value of n_{22} there is a clear minimum near $n_{21} \approx 1.5 - 2.0$, which is in agreement with previous considerations on derivatives. The higher value of n_{22}, the higher uncertainty, which is also in agreement with previous considerations. Concluding Fig. 3b the uncertainty value of the imaginary part of the refractive index rarely exceeds $0.3 \cdot 10^{-6}$. The higher value of n_{21}, the higher uncertainty, and the higher value of n_{22} the higher uncertainty, however, the dependence of the uncertainty on n_{22} is much larger than on n_{21}.

4.3. Uncertainty analysis – Monte Carlo

To further validate the uncertainty analysis we also perform Monte Carlo simulations. The main reason for making such double-check is that approach based on derivatives requires assumptions that derivatives of the higher order than the first one can be neglected, which taking into account the finite value of $u(T_{\text{empty}})$ and $u(R_{\text{empty}})$ can be doubtful, especially in the case when the analyzed function is non-monotonic, has extrema or saddle points. The exact procedure of Monte Carlo simulation is listed below:

a. we defined range of variability of n_{21} and n_{22} to be $1.0 \le n_{21} \le 3.0$ and $0 \le n_{22} \le 20 \cdot 10^{-6}$;
b. for any combination of n_{21} and n_{22} we calculated the reference values (unaffected by noise) of R_{empty} and T_{empty}, this is exactly what is presented in Fig. 2a;
c. for any combination of n_{21} and n_{22} we generated ten thousand pairs of uncorrelated random numbers that have Gaussian distribution, zero mean value, and 0.0025 standard deviation value;
d. for any combination of n_{21} and n_{22} we made a ten thousand numbers long set of noisy values of $R^{\#}_{\text{empty}}$ and $T^{\#}_{\text{empty}}$ by adding random numbers (point c) to the reference values (point b);
e. for any combination of n_{21} and n_{22} we calculated a ten thousands numbers long set of noisy values of $n^{\#}_{21}$ and $n^{\#}_{22}$ using Eq. (11) and noisy values of $R^{\#}_{\text{empty}}$ and $T^{\#}_{\text{empty}}$ (point d) as input;
f. for any combination of n_{21} and n_{22} we calculated a standard deviation of a ten thousand numbers long set of noisy values of $n^{\#}_{21}$ and $n^{\#}_{22}$ (point e); this standard deviation is treated as the uncertainty $u(n_{21})$ and $u(n_{22})$.

Results obtained using Monte Carlo simulations shown in Fig. 3c and Fig. 3d are generally in agreement with the results obtained in an approach based on derivatives, taking into account that they can be a little bit noisy, which results from the limited set of generated and analyzed data set. However, some differences emerge when n_{21} approaches unity.

4.4. Cuvette filled with investigated liquid

Fig. 4. illustrates the results of calculation of transmittance through and reflection from a quartz cuvette ($n_2 = 1.43 + 10^{-7}i$, geometrical details like in Section 3.2) filled with investigated liquid as a function of the real and imaginary part of liquid's refractive index n_3. The range of variability of n_{31} is $1.0 \le n_{31} \le 1.4$ and n_{32} is $0 \le n_{32} \le 50 \cdot 10^{-6}$. The maximal value of n_{31} was set to 1.4 since the real part of the refractive index of a cuvette is 1.43 and results of transmittance and reflectance are overlapping for values of n_{31} lower and higher than 1.43 (there are two sets of values of n_{31} and n_{32} that give the same values of R and T, for example $\{1,0\}$ and $\{2.05,0\}$). Moreover, most aqueous solutions have $n_{31} \approx 1.33$. What is striking in the results illustrated in Fig. 4, especially when compared to the results illustrated in Fig. 2, is the fact that transmittance almost does not depend on the real part of the refractive index of the liquid and is almost linear on the logarithmic scale with the imaginary part of the refractive index of the liquid, which means that T exponentially depends on n_{32}. This answers the question of why even simple approximations allow for a reasonable estimation of the value of the imaginary part of the refractive index. As regards the reflectance from the filled cuvette, it is the most sensitive to n_{31} when n_{31} approaches unity, and is the most sensitive to n_{32} when n_{32} approaches zero. The larger the values of n_{31} and n_{32}, the lower the sensitivity. It is also worth noticing that reflectance from filled cuvette (e.g., with water) is lower than from the empty one regardless of the value of n_{32}, and the reflection further decreases when the absorption increases.

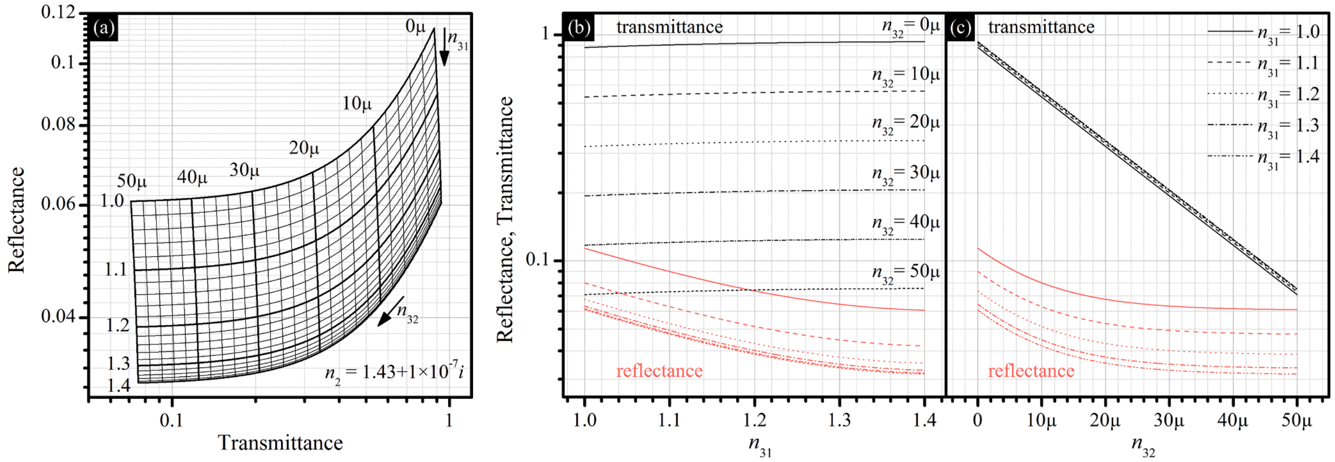

Fig. 4. (a) Dependence of the transmittance and reflectance (T, R) on the real and imaginary parts of the refractive index of an investigated liquid (n_{31}, n_{32}) in a case when the cuvette is characterized by the refractive index $n_2 = 1.43 + 1{\cdot}10^{-7}i$. (b) Dependence of the transmittance T or reflectance R on the real part of the refractive index of an investigated liquid n_{31} for selected values of the imaginary part n_{32}. (c) Dependence of the transmittance T or reflectance R on the imaginary part of the refractive index of an investigated liquid n_{32} for selected values of the real part n_{31}.

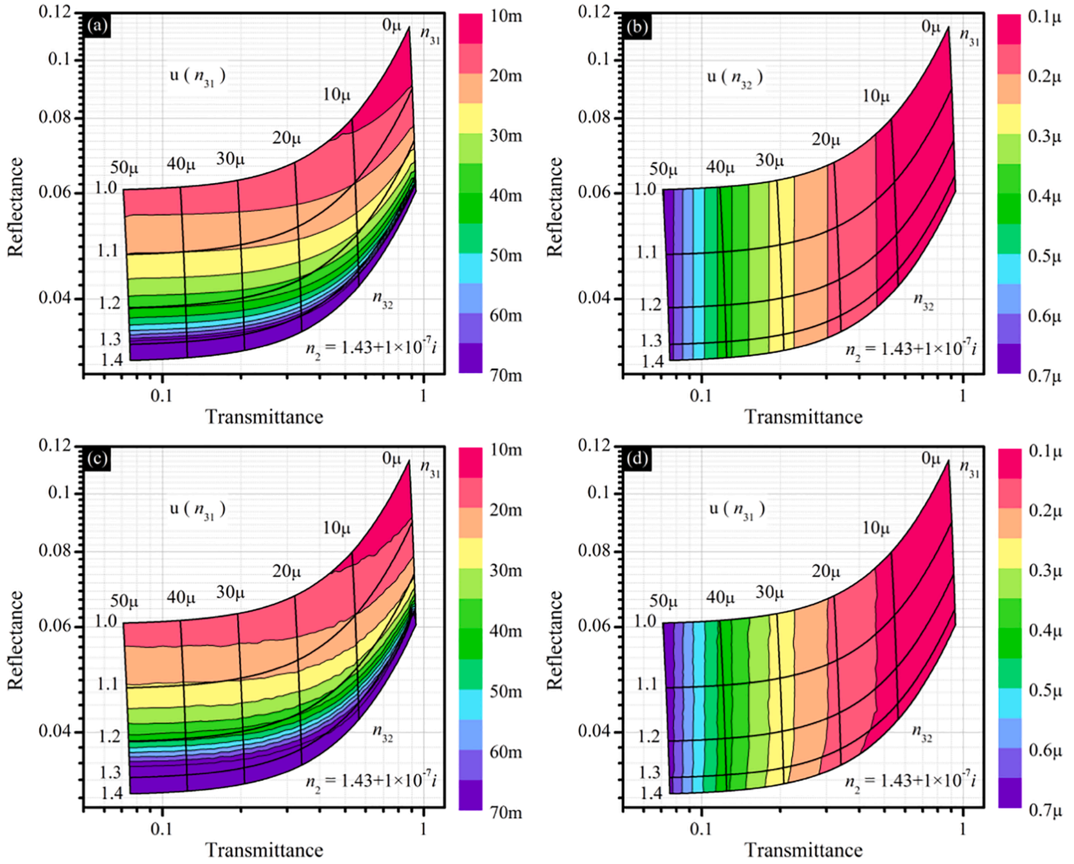

Fig. 5. (a,c) Uncertainty of the real part $\mathrm{u}(n_{31})$ of the refractive index of an investigated liquid and (b,d) uncertainty of the imaginary part $\mathrm{u}(n_{32})$ of the refractive index of an investigated liquid as a function of transmittance and reflectance (T, R) or equivalently as a function of the real and imaginary part of the refractive index of an investigated liquid (n_{31}, n_{32}) in a case when the cuvette is characterized by the refractive index $n_2 = 1.43 + 1{\cdot}10^{-7}i$. The results presented in (a) and (b) are obtained using a derivative approach, the results presented in (c) and (d) are obtained using Monte Carlo simulations. In all cases $\mathrm{u}(T) = \mathrm{u}(R) = 0.0025$.

4.5. Uncertainty analysis – Derivatives

Uncertainty calculations of extracted values of n_{31} and n_{32} are performed in a similar manner that in the case of the empty cuvette, however, are a little bit more complicated since first it is required to calculate n_{21} and n_{22} from R_{empty} and T_{empty} and the second time to calculate n_{31} and n_{32} from R_{filled} and T_{filled} and using the previously found values of n_{21} and n_{22}. The exact expressions used for uncertainty calculations are as follows:

$$
\begin{aligned}
u(n_{31}) &= \sqrt{\left(\frac{\partial n_{31}}{\partial T_{\text{empty}}}\right)^2 u^2\left(T_{\text{empty}}\right) + \left(\frac{\partial n_{31}}{\partial R_{\text{empty}}}\right)^2 u^2\left(R_{\text{empty}}\right) + \left(\frac{\partial n_{31}}{\partial T_{\text{filled}}}\right)^2 u^2\left(T_{\text{filled}}\right) + \left(\frac{\partial n_{31}}{\partial R_{\text{filled}}}\right)^2 u^2\left(R_{\text{filled}}\right)} \\
u(n_{32}) &= \sqrt{\left(\frac{\partial n_{32}}{\partial T_{\text{empty}}}\right)^2 u^2\left(T_{\text{empty}}\right) + \left(\frac{\partial n_{32}}{\partial R_{\text{empty}}}\right)^2 u^2\left(R_{\text{empty}}\right) + \left(\frac{\partial n_{32}}{\partial T_{\text{filled}}}\right)^2 u^2\left(T_{\text{filled}}\right) + \left(\frac{\partial n_{32}}{\partial R_{\text{filled}}}\right)^2 u^2\left(R_{\text{filled}}\right)}
\end{aligned}
\tag{15}
$$

Jacobian matrix and inverse Jacobian matrix have the following forms:

$$
J = \frac{\partial\left(T_{\text{empty}}, R_{\text{empty}}, T_{\text{filled}}, R_{\text{filled}}\right)}{\partial(n_{21}, n_{22}, n_{31}, n_{32})} = \begin{pmatrix}
\frac{\partial T_{\text{empty}}}{\partial n_{21}} & \frac{\partial T_{\text{empty}}}{\partial n_{22}} & \frac{\partial T_{\text{empty}}}{\partial n_{31}} & \frac{\partial T_{\text{empty}}}{\partial n_{32}} \\
\frac{\partial R_{\text{empty}}}{\partial n_{21}} & \frac{\partial R_{\text{empty}}}{\partial n_{22}} & \frac{\partial R_{\text{empty}}}{\partial n_{31}} & \frac{\partial R_{\text{empty}}}{\partial n_{32}} \\
\frac{\partial T_{\text{filled}}}{\partial n_{21}} & \frac{\partial T_{\text{filled}}}{\partial n_{22}} & \frac{\partial T_{\text{filled}}}{\partial n_{31}} & \frac{\partial T_{\text{filled}}}{\partial n_{32}} \\
\frac{\partial R_{\text{filled}}}{\partial n_{21}} & \frac{\partial R_{\text{filled}}}{\partial n_{22}} & \frac{\partial R_{\text{filled}}}{\partial n_{31}} & \frac{\partial R_{\text{filled}}}{\partial n_{32}}
\end{pmatrix},
$$

$$
J^{-1} = \frac{\partial(n_{21}, n_{22}, n_{31}, n_{32})}{\partial\left(T_{\text{empty}}, R_{\text{empty}}, T_{\text{filled}}, R_{\text{filled}}\right)} = \begin{pmatrix}
\frac{\partial n_{21}}{\partial T_{\text{empty}}} & \frac{\partial n_{21}}{\partial R_{\text{empty}}} & \frac{\partial n_{21}}{\partial T_{\text{filled}}} & \frac{\partial n_{21}}{\partial R_{\text{filled}}} \\
\frac{\partial n_{22}}{\partial T_{\text{empty}}} & \frac{\partial n_{22}}{\partial R_{\text{empty}}} & \frac{\partial n_{22}}{\partial T_{\text{filled}}} & \frac{\partial n_{22}}{\partial R_{\text{filled}}} \\
\frac{\partial n_{31}}{\partial T_{\text{empty}}} & \frac{\partial n_{31}}{\partial R_{\text{empty}}} & \frac{\partial n_{31}}{\partial T_{\text{filled}}} & \frac{\partial n_{31}}{\partial R_{\text{filled}}} \\
\frac{\partial n_{32}}{\partial T_{\text{empty}}} & \frac{\partial n_{32}}{\partial R_{\text{empty}}} & \frac{\partial n_{32}}{\partial T_{\text{filled}}} & \frac{\partial n_{32}}{\partial R_{\text{filled}}}
\end{pmatrix}
\tag{16}
$$

The results of uncertainty calculations are shown in Fig. 5a and Fig. 5b. Concluding Fig. 5a one can see that the uncertainty of the real part of the liquid's refractive index depends mostly on the value of the reflectance, whereas the imaginary part of the liquid's refractive index depends mostly on the transmittance value. It is a qualitatively different result than in the case of an empty cuvette. Moreover, the scale of changes of absolute values of uncertainties is more than twice as large as in the case of the empty cuvette, which however, is quite intuitive.

4.6. Uncertainty analysis – Monte Carlo

Similarly like in the case of the empty cuvette, we calculated what the uncertainties of the extracted parameters of investigated liquid are using Monte Carlo simulations. We note, however, that here we made both the transmittance and reflectance from the empty cuvette ($R^{\#}_{\text{empty}}$, $T^{\#}_{\text{empty}}$) and from the cuvette filled with liquid ($R^{\#}_{\text{filled}}$, $T^{\#}_{\text{filled}}$) noisy. The results are shown in Fig. 5c and Fig. 5d, and are generally in agreement with results obtained in the approach based on derivatives, similarly like in the case of the empty cuvette, and start to differ when n_{31} approaches 1.4, which is close to $n_{21} = 1.43$.

4.7. Cuvette dimensions

In order to calculate the real and imaginary parts of the refractive indices of the quartz cuvette and the investigated liquid one has to provide values of the transmittance and reflectance from the empty and filled cuvette. However, there are two more values which have to be known before performing the final calculations and which are related to the geometrical dimensions of the cuvette: the cuvette wall thickness d_2 and the distance between cuvette walls d_3. These two geometrical parameters affect the absorption of the light when passing through media 2 (quartz) because $A_2 = e^{-\alpha_2 \cdot d_2}$, and media 3 (investigated liquid) because $A_3 = e^{-\alpha_3 \cdot d_3}$. Thus $u(d_2)$ an $u(d_3)$ will contribute primarily the uncertainties of the imaginary parts of the refractive indices: $u(n_{22})$ and $u(n_{32})$ whereas the uncertainties of the real parts of the refractive indices will be affected only slightly.

Formally, to calculate the uncertainties of the real and imaginary parts of the refractive index of the empty cuvette we can use the following formulas:

$$
\begin{aligned}
u(n_{21}) &= \left|\frac{\partial n_{21}}{\partial d_2}\right| \cdot u(d_2) = \left|\frac{\partial n_{21}}{\partial T_{\text{empty}}}\frac{\partial T_{\text{empty}}}{\partial d_2} + \frac{\partial n_{21}}{\partial R_{\text{empty}}}\frac{\partial R_{\text{empty}}}{\partial d_2}\right| \cdot u(d_2) \\
u(n_{22}) &= \left|\frac{\partial n_{22}}{\partial d_2}\right| \cdot u(d_2) = \left|\frac{\partial n_{22}}{\partial T_{\text{empty}}}\frac{\partial T_{\text{empty}}}{\partial d_2} + \frac{\partial n_{22}}{\partial R_{\text{empty}}}\frac{\partial R_{\text{empty}}}{\partial d_2}\right| \cdot u(d_2)
\end{aligned}
\tag{17}
$$

Such indirect calculations are required because there is no analytical expression linking n_{21} and n_{22} with d_2 but there are analytical expressions for $\partial T_{\text{empty}}/\partial d_2$ and for $\partial R_{\text{empty}}/\partial d_2$ as well as for the remaining partial derivatives as was demonstrated in Section 4.2 Eq. (14). We also note that in the case of lossless medium 1 (air) there is no influence of the $u(d_3)$ on $u(n_{21})$ and $u(n_{22})$ since $\alpha_3 = 0$ and therefore $A_3 = e^{-\alpha_3 \cdot d_3} = 1$ despite any value of d_3. Fig. 6a and b illustrate results of calculations of $u(n_{21})$ and $u(n_{22})$ for an arbitrary value of $u(d_2)$ that equals 0.01 mm. As can be seen the contribution from the geometrical dimension uncertainty to the uncertainty of the real part of the refractive index of the empty cuvette is completely negligible, but in the case of the imaginary part of the refractive index contribution from the geometrical dimension uncertainty is significant and approximately equals $u(n_{22}) \approx u(d_2)/d_2 \cdot n_{22} = 0.008 \cdot n_{22}$.

Calculation of the uncertainties of the real and imaginary parts of the refractive index of the investigated liquid can be performed in a similar manner than previously taking into account that this time $u(n_{31})$ and $u(n_{32})$ will depend both on $u(d_2)$ an $u(d_3)$. Formally:

$$
\begin{aligned}
u(n_{31}) &= \sqrt{\left(\frac{\partial n_{31}}{\partial d_2}\right)^2 u^2(d_2) + \left(\frac{\partial n_{31}}{\partial d_3}\right)^2 u^2(d_3)} = \sqrt{\left(\frac{\partial n_{31}}{\partial T_{\mathrm{empty}}}\frac{\partial T_{\mathrm{empty}}}{\partial d_2} + \frac{\partial n_{31}}{\partial R_{\mathrm{empty}}}\frac{\partial R_{\mathrm{empty}}}{\partial d_2} + \frac{\partial n_{31}}{\partial T_{\mathrm{filled}}}\frac{\partial T_{\mathrm{filled}}}{\partial d_2} + \frac{\partial n_{31}}{\partial R_{\mathrm{filled}}}\frac{\partial R_{\mathrm{filled}}}{\partial d_2}\right)^2 u^2(d_2) + \left(\frac{\partial n_{31}}{\partial T_{\mathrm{empty}}}\frac{\partial T_{\mathrm{empty}}}{\partial d_3} + \frac{\partial n_{31}}{\partial R_{\mathrm{empty}}}\frac{\partial R_{\mathrm{empty}}}{\partial d_3} + \frac{\partial n_{31}}{\partial T_{\mathrm{filled}}}\frac{\partial T_{\mathrm{filled}}}{\partial d_3} + \frac{\partial n_{31}}{\partial R_{\mathrm{filled}}}\frac{\partial R_{\mathrm{filled}}}{\partial d_3}\right)^2 u^2(d_3)} \\
u(n_{32}) &= \sqrt{\left(\frac{\partial n_{32}}{\partial d_2}\right)^2 u^2(d_2) + \left(\frac{\partial n_{32}}{\partial d_3}\right)^2 u^2(d_3)} = \sqrt{\left(\frac{\partial n_{32}}{\partial T_{\mathrm{empty}}}\frac{\partial T_{\mathrm{empty}}}{\partial d_2} + \frac{\partial n_{32}}{\partial R_{\mathrm{empty}}}\frac{\partial R_{\mathrm{empty}}}{\partial d_2} + \frac{\partial n_{32}}{\partial T_{\mathrm{filled}}}\frac{\partial T_{\mathrm{filled}}}{\partial d_2} + \frac{\partial n_{32}}{\partial R_{\mathrm{filled}}}\frac{\partial R_{\mathrm{filled}}}{\partial d_2}\right)^2 u^2(d_2) + \left(\frac{\partial n_{32}}{\partial T_{\mathrm{empty}}}\frac{\partial T_{\mathrm{empty}}}{\partial d_3} + \frac{\partial n_{32}}{\partial R_{\mathrm{empty}}}\frac{\partial R_{\mathrm{empty}}}{\partial d_3} + \frac{\partial n_{32}}{\partial T_{\mathrm{filled}}}\frac{\partial T_{\mathrm{filled}}}{\partial d_3} + \frac{\partial n_{32}}{\partial R_{\mathrm{filled}}}\frac{\partial R_{\mathrm{filled}}}{\partial d_3}\right)^2 u^2(d_3)}
\end{aligned}
\tag{18}
$$

In the case of the cuvette filled with liquid, similarly like in the case of empty cuvette, each of the partial derivatives $\partial n_{31}/\partial d_2$, $\partial n_{31}/\partial d_3$, $\partial n_{32}/\partial d_2$, $\partial n_{32}/\partial d_3$ has to be expanded into a sum of four products, which part of can be analytically calculated and part has to be calculated using inverse Jacobian matrix, as described in Section 4.5 Eq. (16). Fig. 6c and d illustrate results of calculations of $u(n_{31})$ and $u(n_{32})$ for an arbitrary value of $u(d_2)$ and $u(d_3)$ that equal 0.01 mm. As can be seen the contribution from the geometrical dimension uncertainty to the uncertainty of the real part of the refractive index of the investigated liquid is completely negligible, but in the case of the imaginary part of the refractive index contribution from the geometrical dimension uncertainty is significant and approximately equals $u(n_{22}) \approx u(d_3)/d_3 \cdot n_{22} = 0.005 \cdot n_{22}$.

Results of the Monte Carlo simulations (not shown) are in agreement with results obtained in the approach based on derivatives, similarly like in the case of the empty and filled cuvette.

5. Comparison of the rigorous model with popular approximations

Rigorous calculations of optical properties of investigated liquid require values of both the transmittance and reflectance. Neglection of the reflectance, which is typical for popular approximations, makes the

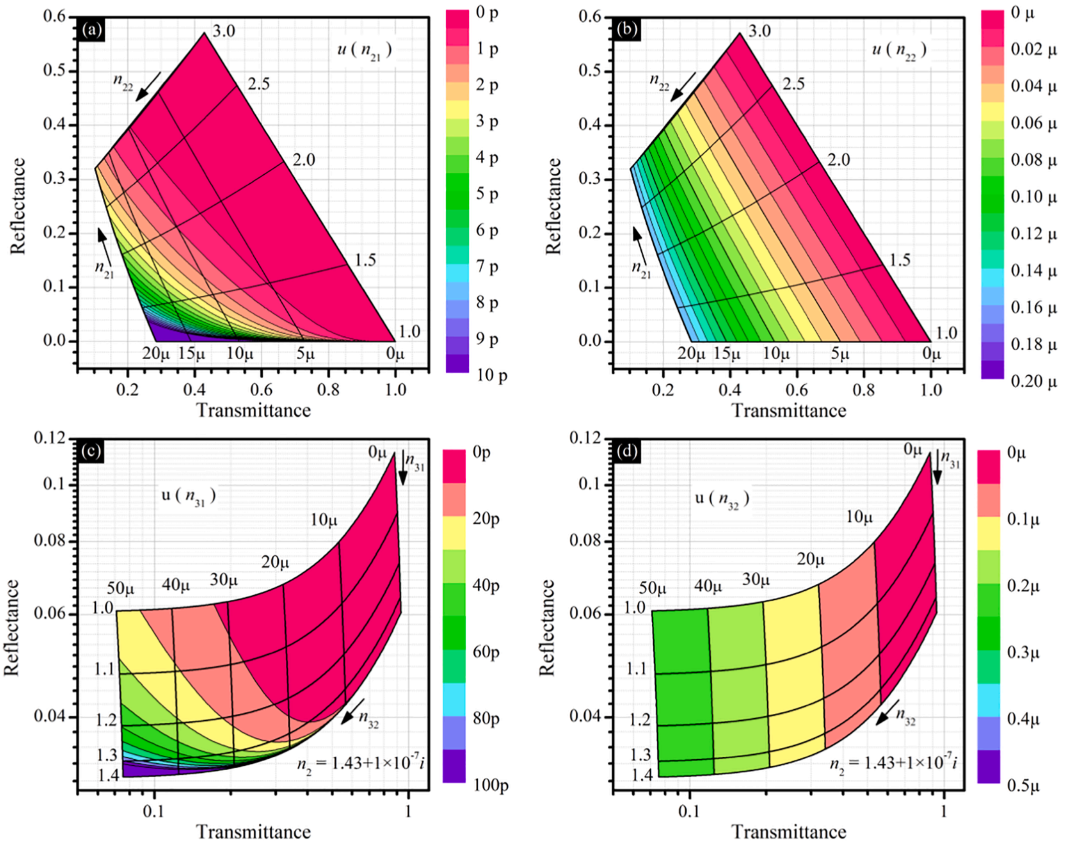

Fig. 6. Contribution of the geometrical uncertainty to (a) the uncertainty of the real part $\mathrm{u}(n_{21})$ of the refractive index of a cuvette and (b) the uncertainty of the imaginary part $\mathrm{u}(n_{22})$ of the refractive index of a cuvette as a function of transmittance and reflectance (T, R) or equivalently as a function of the real and imaginary parts of the refractive index of a cuvette (n_{21}, n_{22}) in a case when the cuvette is empty (filled with air). Contribution of the geometrical uncertainty to (c) the uncertainty of the real part $\mathrm{u}(n_{31})$ of the refractive index of an investigated liquid and (d) the uncertainty of the imaginary part $\mathrm{u}(n_{32})$ of the refractive index of an investigated liquid as a function of transmittance and reflectance (T, R) or equivalently as a function of the real and imaginary part of the refractive index of an investigated liquid (n_{31}, n_{32}) in a case when the cuvette is characterized by the refractive index $n_2 = 1.43 + 1 \cdot 10^{-7} i$. In all cases $\mathrm{u}(d_2) = \mathrm{u}(d_3) = 0.01\mathrm{mm}$.

calculation of the real part of the liquid refractive index impossible and introduces systematic errors to the calculations of the absorption coefficient. However, having an rigorous optical model we are able to calculate these systematic errors exactly and further compare them with the uncertainties. Such calculations will answer the question how accurate are the popular approximation and if they are valid.

5.1. Differences in the measurement procedure

Most of the commercially available spectrophotometric setups measures only the transmittance or relative transmittance. For example, in a double-beam spectrophotometers the light beam is divided into two same intensity beams. The first of the beams, the reference beam, passes undisturbed (there is no sample in the optical path) or through the reference sample (empty cuvette, cuvette filled with non-absorbing solvent). The second of the beams passes through the investigated sample (cuvette filled with the investigated liquid). Thus, the absorbance of the investigated solute (let's assume we want to use the Beer-Lambert law) can be easily estimated using the following formulas:

$$A_{\text{solute}} = \varepsilon_{\text{solute}}\cdot c_{\text{solute}}\cdot d = \alpha_{\text{solute}}\cdot d \approx \begin{cases} -\log_{10} T_{\text{filled}}(c_{\text{solute}})/T_0 & \text{approximaton 1} \\ -\log_{10} T_{\text{filled}}(c_{\text{solute}})/T_{\text{empty}} & \text{approximaton 2} \\ -\log_{10} T_{\text{filled}}(c_{\text{solute}})/T_{\text{filled}}(c_{\text{solute}}=0) & \text{approximaton 3} \end{cases}, \tag{19}$$

where T_0 is the transmittance value in a case when there is no sample in the optical path of the reference beam, T_{empty} is the transmittance value in a case when there is an empty cuvette in the optical path of the reference beam, and $T_{\text{filled}}(c_{\text{solute}} = 0)$ is the transmittance value in a case when there is a cuvette filled with non-absorbing solvent in the optical path of the reference beam. Other words, these three reference values – T_0, T_{empty} and $T_{\text{filled}}(c_{\text{solute}} = 0)$, are used to normalize the transmittance through the cuvette filled with investigated liquid $T_{\text{filled}}(c)$. Such a normalization is a big advantage of the modern method for estimating the absorbance of the investigated solute since it makes the method calibration-free (because the absolute value of the transmittance is not required). The main disadvantage is that the modern method tries to describe properties of an optical system that is parametrized by four independent numbers – n_{21}, n_{22}, n_{31}, and n_{32}, only by two measured values, which undoubtedly leads to some undefined systematic errors.

Presented in this work rigorous model does not introduce mentioned above systematic errors, because it describes properties of an optical system parametrized by four independent numbers – n_{21}, n_{22}, n_{31}, and n_{32}, by four measured values – T_{empty}, R_{empty}, T_{filled}, and R_{filled}. And this is an advantage. The main disadvantage of our approach is a more advanced measurement procedure, which requires knowledge of the absolute values of the reflection from empty R_{empty} and filled cuvette R_{filled}, which requires proper calibration (e.g. measurements using a material with precisely known parameters).

5.2. Differences in the definitions of the absorption coefficient

We also would like to point out that there is some ambiguity in the literature concerning the definition of absorbance and absorption coefficient. Formally, the absorbance A and absorption coefficient α are often defined using two similar expressions:

$$\begin{aligned} A' &= -\log_{10} T = \varepsilon'\cdot c\cdot d = \alpha'\cdot d \Leftrightarrow T = 10^{-A'} = 10^{-\varepsilon'\cdot c\cdot d} = 10^{-\alpha'\cdot d} \\ A'' &= -\ln T = \varepsilon''\cdot c\cdot d = \alpha''\cdot d \Leftrightarrow T = e^{-A''} = e^{-\varepsilon''\cdot c\cdot d} = e^{-\alpha''\cdot d} \end{aligned}, \tag{20}$$

where $A'' = A'\cdot\ln 10$ and $\alpha'' = \alpha'\cdot\ln 10$. This ambiguity is not problematic but requires consequence in using chosen definition. Typically in spectrophotometry of liquids the decadic absorption coefficient α' is used, whereas in papers more focused on physics or optics the Napierian absorption coefficient α'' is a more popular choice. In this work we used the latter definition since we start our consideration from the definition of the electric field intensity of an electromagnetic plane wave that travels along x-axis – see Eq. (2).

5.3. Calculations of the systematic errors

The exact procedure of calculation of systematical errors introduced

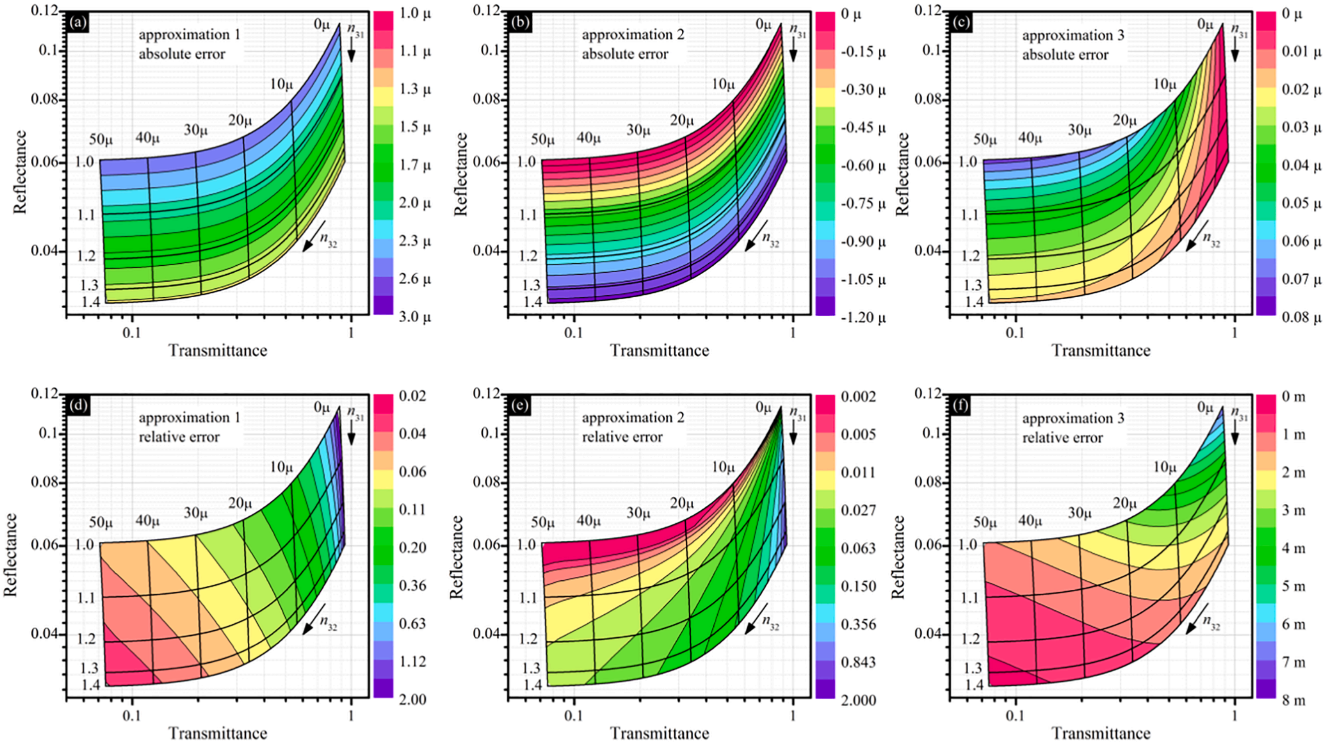

Fig. 7. Values of the absolute $\epsilon^{\text{a1,2,3}}_{\text{n32}}$ and relative $\eta^{\text{a1,2,3}}_{\text{n32}}$ systematic errors introduced into calculations of n_{32} by three approximations popular in the literature.

into the calculation of attenuation coefficient using three popular approximations consists of four points:

a. we defined the range of variability of n_{31} and n_{32} to be $1.0 \leq n_{31} \leq 1.4$ and $0 \leq n_{32} \leq 50 \cdot 10^{-6}$; we assumed that d_3 – optical path length in liquid equals 2 mm;
b. for any combination of n_{31} and n_{32} we calculated the values of R_{filled} and T_{filled}, assuming $n_2 = 1.43 + 10^{-7}i$;
c. for any combination of n_{31} and n_{32} we calculated the values of n_{32} using three popular approximations:
   - $n_{32}^{\mathrm{a1}} = -\frac{\lambda}{4\pi d} \cdot \ln T_{\mathrm{filled}}$
   - $n_{32}^{\mathrm{a2}} = -\frac{\lambda}{4\pi d} \cdot \ln T_{\mathrm{filled}} / T_{\mathrm{empty}}$
   - $n_{32}^{\mathrm{a3}} = -\frac{\lambda}{4\pi d} \cdot \ln T_{\mathrm{filled}} / T_{\mathrm{filled}, n_{32}=0}$
d. for any combination of n_{31} and n_{32} we calculated systematic error twice, first time as an absolute error $\epsilon_{\mathrm{n32}}^{\mathrm{a1,2,3}} = n_{32}^{\mathrm{a1,2,3}} - n_{32}$ and the second time as a relative error $\eta_{\mathrm{n32}}^{\mathrm{a1,2,3}} = \frac{n_{32}^{\mathrm{a1,2,3}} - n_{32}}{n_{32}}$.

The results of all calculations are depicted in Fig. 7. As can be seen in Fig. 7a the values of the absolute error $\epsilon_{\mathrm{n32}}^{\mathrm{a1}}$ introduced into the calculations of n_{32} by the first approximation, in which no renormalization of transmittance is made, is in the $1 \cdot 10^{-6}$–$3 \cdot 10^{-6}$ range, and depends mostly on the real part of liquid's refractive index. The values of the relative systematic error $\eta_{\mathrm{n32}}^{\mathrm{a1}}$ are depicted in Fig. 7d, and as can be seen $\eta_{\mathrm{n32}}^{\mathrm{a1}}$ exceeds 200% for low values of n_{32}, equal approximately 10%–20% for moderate values of n_{32} understood as included in the $10 \cdot 10^{-6}$–$20 \cdot 10^{-6}$ range, and achieve few percents for the highest values of n_{32}. The relative systematic error depends more on n_{32} than n_{31}. Comparing values of the absolute systematic error $\epsilon_{\mathrm{n32}}^{\mathrm{a1}}$ depicted in Fig. 7a with values of uncertainties $u(n_{32})$ depicted in Fig. 5b, we see that the values of $\epsilon_{\mathrm{n32}}^{\mathrm{a1}}$ are always above values of $u(n_{32})$, which means that the results obtained using the first of the popular approximations are significantly affected by the imperfections of this approximation. We note that the contribution to this systematic error is not only the neglect of the existence of the cuvette but also the neglect of all reflections in the considered optical system. In other words, the first model assumes absorption by a nonreflecting slab of liquid.

In Fig. 7b one can find results of calculations of the values of the absolute error introduced into calculations of n_{32} using the second approximation, in which the transmittance is normalized to the transmittance of the empty cuvette. The values of $\epsilon_{\mathrm{n32}}^{\mathrm{a2}}$ are in the $-1.2 \cdot 10^{-6}$–$0 \cdot 10^{-6}$ range, depend mostly on n_{31}, and contrary to the first model for which values of n_{32} are overestimated, here almost all values are underestimated. It results from the fact that the reflection from the empty cuvette is larger than that from the filled one. The values of the module of the relative systematic error $\eta_{\mathrm{n32}}^{\mathrm{a2}}$ are depicted in Fig. 7e, and as can be seen $\eta_{\mathrm{n32}}^{\mathrm{a2}}$ exceed 200% for low values of n_{32}, do not exceed 10% for moderate values of n_{32}, and drop below one percent for the highest values of n_{32}. The relative systematic error depends both on n_{31} and n_{32}, and for low values of n_{31} has the lowest value. Comparing the values of the absolute systematic error $\epsilon_{\mathrm{n32}}^{\mathrm{a2}}$ depicted in Fig. 7b with the values of uncertainties $u(n_{32})$ depicted in Fig. 5b, we see that values of $\epsilon_{\mathrm{n32}}^{\mathrm{a2}}$ are above values of $u(n_{32})$ for higher values of n_{31}, $\epsilon_{\mathrm{n32}}^{\mathrm{a2}}$ are below values of $u(n_{32})$ for low values of n_{31} and high values of n_{32}, and $\epsilon_{\mathrm{n32}}^{\mathrm{a2}}$ are of the same order than $u(n_{32})$ for low values of n_{31} and low values of n_{32}. This means that the results obtained using the second of the popular approximations can be also significantly affected by the imperfections of this approximation, particularly for aqueous solutions ($n_{31} \approx 1.33$) the considered approximation is not optimal.

In Fig. 7c one can find results of the calculations of the values of the absolute error introduced into calculations of n_{32} using the third approximation, in which transmittance is normalized to the transmittance of the cuvette filled with a liquid with the same n_{31} but with no absorption. Typically it is a pure (non-absorbing) solvent. The values of $\epsilon_{\mathrm{n32}}^{\mathrm{a3}}$ do not exceed $0.08 \cdot 10^{-6}$, and depend both on n_{31} and n_{32}. The values of $\eta_{\mathrm{n32}}^{\mathrm{a3}}$ do not exceed 0.008 (0.8%) and also depend both on n_{31} and n_{32}. Comparing the values of the absolute systematic error $\epsilon_{\mathrm{n32}}^{\mathrm{a3}}$ depicted in Fig. 7c with values of uncertainties $u(n_{32})$ depicted in Fig. 5b, we see that the values of $\epsilon_{\mathrm{n32}}^{\mathrm{a3}}$ are significantly below the values of $u(n_{32})$, which means that the third approximation can be used for reliable calculations of n_{32}, assuming measurement uncertainty of the transmittance and reflectance to be 0.0025.

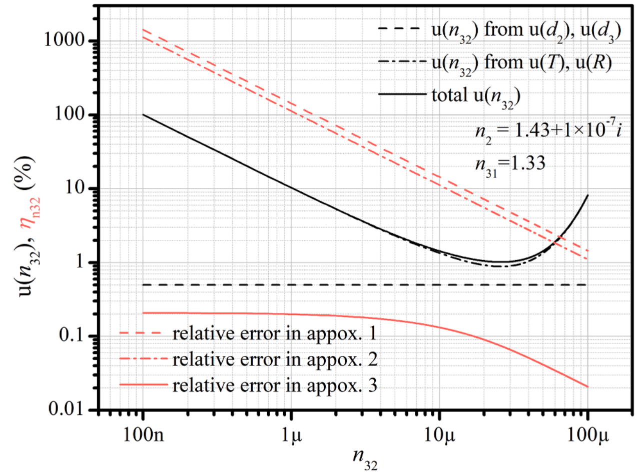

Fig. 8. Comparison of different contribution to the uncertainty of the imaginary part of the liquids refractive index $\mathrm{u}(n_{32})$ with relative systematic errors $\eta_{\mathrm{n32}}^{\mathrm{a1,2,3}}$ introduced into calculations of n_{32} by three approximations popular in the literature for quartz cuvette and aqueous solutions $n_{31} = 1.33$.

5.4. Aqueous solutions

At the end it is worth to compare different contribution to the uncertainty of the imaginary part of the liquids refractive index $\mathrm{u}(n_{32})$ with relative systematic errors $\eta_{\mathrm{n32}}^{\mathrm{a1,2,3}}$ introduced into calculations of n_{32} by three approximations popular in the literature for quartz cuvette characterized by $n_2 = 1.43 + 1 \cdot 10^{-7}i$ and in the case of aqueous solutions $n_{31} = 1.33$. As can be seen in Fig. 8 the relative systematic errors introduced by the first and second approximations to n_{32} value are typically one order of magnitude larger than the measurement uncertainty $u(n_{32})$, except for the largest values of n_{32}, but the relative systematic errors introduced by the third approximations to n_{32} value are at least one order of magnitude smaller than the measurement uncertainty $u(n_{32})$.

6. Conclusions

Development of a rigorous optical model of a cuvette filled with liquid allowed us for validation of three popular approximations used in the literature for calculation of absorption, or equivalently of n_{32}. We demonstrated what the differences in obtained results are when employing different approximations, we compared them with estimated uncertainties, and we showed that the third approximation yields very accurate results, i.e. systematic errors introduced by this method can be at least a few times smaller than the measurement uncertainties. We point out that the collation of the systematic errors with the uncertainties is crucial in this conclusion since only this way one is allowed to say what a significant difference is and what difference can be neglected. We also note that our conclusions are not absolute, i.e. they are related to our experimental setup and to measurement uncertainties of R and T that equal 0.0025; for other experimental setups characterized by different values of reflectance and transmittance uncertainties conclusions can be different, e.g. the difference between our strict model and the most precise models from the literature can be significant, thus invalidating the closest approximation.

CRediT authorship contribution statement

Jarosław Judek: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing. **Konrad Wilczyński:** Investigation, Writing - review & editing. **Jerzy K. Piotrowski:** Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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