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Determination of the refractive index of microparticles by utilizing light dispersion properties of the particle and an immersion liquid

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ABSTRACT

The knowledge of the refractive index of a particle is important in sensing and imaging applications, e.g., in biology, medicine and process industry. The refractive index of tiny solid particles such as microsize particles can be determined by the so-called liquid immersion technique. This study deals with three different types of interrogation methods to get the refractive index of a particle in a liquid matrix. These methods utilize thermo-optical properties and wavelength-dependent refractive index of the particle and the immersion liquids, as well as, the classical method using a set of in advance prepared set of immersion liquids with different refractive indices. The emphasis is on a method to get especially the wavelength-dependent refractive index of microparticles and exploiting different wavelength-dependences of immersion liquid and a solid particle because identification of a particle is more reliable if the refractive index of the particle is known at several wavelengths. In this study glycerol–water mixtures served as immersion liquids to obtain the refractive index of CaF_2 at several discrete wavelengths in the spectral range 200–500 nm. The idea is to find the maximum value of light transmission of suspension by scanning the wavelength of a commercial spectrophotometer. The light dispersion-based method is suggested as a relatively easy, economic and fast method to determine the refractive index of a particle by a spectrophotometer at several wavelengths of light. The accuracy of the detection of the refractive index is suggested to be better than ± 0.005 refractive index units.

1. Introduction

One significant optical property of particles is the refractive index (n) which is an intrinsic material property. Information on the refractive index of particles is important e.g. with pigments in the paper industry (rheology of slurries), since pigments strongly affect the optical properties of paper such as brightness, gloss, smoothness, whiteness, opacity and generate better printing surface properties [1]. Furthermore, the accurate determination of the refractive index plays an important role in material research, for example, to estimate the purity of a material.

Several methods have been developed for the determination of refractive index of a solid material. For example, refractive index of solid particles can be estimated by observing light scattering at different scattering angles [2]. Unfortunately, there is no rigorous scattering theory currently that would provide accurate refractive index of a particle having an irregular shape and unknown size/volume in suspension. Fortunately, the advantages of liquid immersion methods are that the shape and size of a particle are not issues [3]. The immersion method is well-known from optical microscopy. The idea of the liquid immersion method, also in the case of irregular particles, is to find a liquid or a condition of the sample so that the refractive index of the liquid equals to the refractive index of the solid micro-and/or nanosize particle. The liquid immersion method has been widely used, for instance, in the determination of the refractive index of minerals [4,5], heat treated wood [6], paper [7], clearing of tissue [8], biological particles [9] and cosmetics [10]. In microscopy, one typically uses a set of immersion liquids, and the determination of the refractive index is based on the measurer's visual observation of an individual particle. Preparation of samples using a set of immersion liquids with different refractive indices is usually time-consuming and the accuracy and reliability of the results can be low if the accuracy is based on the relatively low accuracy of the refractive index values given by the manufacturer of a commercial liquid. However, a better accuracy is obtained by measuring commercial liquid with a refractometer.

Wiederseiner et al. have written a review article on refractive-index matching with different techniques and provide an extensive source of references of the field [11]. Quite often the refractive

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index matching is based on the use of a fixed wavelength of a laser as a light source, because the wavelength is exactly known and relatively cheap lasers are available. However, refractive index data of the immersion liquids and liquids in general are typically given only for the wavelength of sodium light (the relevant device, namely Abbe refractometer, is operating at such a fixed wavelength). Furthermore, in many cases such as in [12], the data of the wavelength-dependent refractive index of liquids and solid media are obtained from the manufacturers and with relatively low accuracy. These are drawbacks if one wants to get the refractive index of the solid particle at several wavelengths and with higher accuracy in the frame of the immersion liquid method.

We have measured the refractive index of isotropic, and also effective refractive index of anisotropic (birefringent) particles, as reported in references [13,14], by using procedures developed for the traditional liquid immersion method, which can be even utilized in the assessment of the particle concentration of a slurry. We have also determined the refractive index of modified inorganic and organic pigments in order to nanotailor new optical properties to host pigments of paper products [15].

The light dispersion and thermo-optic coefficients of liquid and a solid medium are typically different from each other and therefore it is, in principle, possible to find a matching condition in which the refractive indices of a solid material and a liquid are the same [11]. One object of this study is to get the refractive index of a particle population by exploiting the dispersion and thermo-optic properties of materials involved and by using a common laboratory apparatus, a spectrophotometer in the transmission measurement mode. To our knowledge, this is the first time when the dispersion properties of materials involved in immersion studies have been used to obtain the refractive index of a particle at multiple wavelengths. In that sense, this study differs from Ref. [11]. Another object is to compare qualitatively different immersion liquid techniques against each other.

2. Material and methods

In the literature [11] three different phenomena related to immersion liquid methods to assess the refractive index of particles are described. Typically, a microscope is used to detect the refractive index of solid particles using transmission of light in the context of the immersion techniques. Here we assume that all liquids involved are colorless. Main differences of the methods are characterized as follows.

2.1. The immersion liquid set method

The refractive index sweep is realized simply by mixing two or several different liquids together (manually or automatically) and measuring the light transmittance or scattering from a set of immersion liquids with different refractive indices. When the index matching occurs between the immersion liquid and the particle the transmittance attains a maximum or light scattering a minimum value. Measurements are performed using a commercial spectrophotometer at constant temperature and for a fixed wavelength of a light source.

2.2. The temperature method

The refractive index of a medium depends on the temperature, and the phenomenon can be described by the thermo-optic coefficient dn/dt, where t is the temperature in Celsius degrees. The refractive index sweep for index matching is obtained by changing the temperature of the sample. The wavelength is kept constant. Here, knowledge of different thermo-optic properties for a liquid and a particle is desirable. At a certain temperature there will occur index matching and hence the maximum transmittance for the fixed wavelength. A spectrophotometer can be used for measurement of transmittance.

2.3. The wavelength method

This method, using the so-called Christiansen effect [12,16], is based on the fact that all materials have their intrinsic dispersion $n(\lambda)$ characteristics. The refractive indices of the immersion liquid and a particle may equal at certain wavelength λ. This condition can be observed from the transmission spectrum and particularly from the spectral location of the maximum transmittance. One (refractive index obtained at one wavelength) or several (refractive index data obtained at several wavelengths) immersion liquids can be chosen among a set of immersion liquid candidates and the measurement temperature should be fixed. A spectrophotometer can be utilized to get the refractive index of a particle population by scanning the wavelength of the probe light and observing maximum transmittance for a certain wavelength.

To prepare a set of immersion liquids to obtain test liquids with a range of refractive index values, often two liquids have to be mixed together. Let us assume that an immersion liquid is a mixture of two liquids 1 and 2. Conventionally the determination of the refractive index n_{21} of the immersion liquid is based on the measurement of the volumes V_1 and V_2 of two liquids, *a priori* knowledge of their refractive indices n_1 and n_2, and the use of the Arago–Biot formula [10,17]

$$n_{21} = \frac{V_1 n_1 + V_2 n_2}{V_1 + V_2}. \quad (1)$$

The refractive indices of pure liquids 1 and 2 (n_1 and n_2) were measured using a commercial Abbe refractometer (Atago RX5000) at the wavelength 589 nm. According to the manufacturer the refractive index accuracy of the device was ± 0.00004. Eq. (1) was exploited in the preparation process of immersion liquids. Finally, the refractive index of liquid mixture was checked by the refractometer. The transmittance was measured using a Hitachi U-3300 spectrophotometer.

The solid medium in this study was CaF_2 powder, which is a product of Merck. The purity of CaF_2 sample is higher than 97% (according to the product description it contains small amounts of Fe, Mg, Na and SiO_4). The average diameter of a particle, determined by a sedimentation technique (SediGraph III), was 17.3 μm. Liquids used in tests were purified water and glycerol (J. T. Barker Company), and they were mixed together to obtain a set of immersion liquids with slightly different refractive indices. Transmission measurements utilized the sample path length of 10 mm.

3. Result and discussion

Firstly, for the immersion liquid set method the transmission of CaF_2 suspended in water–glycerol mixtures was measured. The refractive index of the immersion liquid varied from 1.330 to 1.470 with a step interval of 0.005 refractive index unit. The total number of samples was 30. Measurements were performed with a spectrophotometer at room temperature, and probe wavelength 589 nm was chosen. Because of convenience in all cases of the immersion set method the mass concentration of CaF_2 was chosen, 4 g in a total suspension volume of 100 ml, which is an amount that is easy and accurate to measure. More importantly, such a choice provides a wide enough full width at half maximum of the transmittance curve presented as a function of the refractive index. This in turn improves finding the correct value of the index match in data fitting that is explained below. Transmittance curve

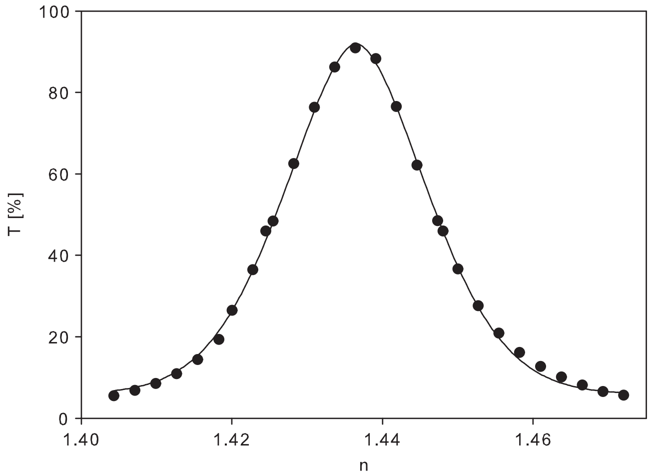

Fig. 1. The immersion liquid set method: transmission data as a function of the refractive index (filled circles) at 589 nm. The solid line is a Gaussian line used for fitting the experimental data.

Table 1
Refractive index of CaF_2 obtained from immersion methods and literature.

λ(nm)	*n*	*n*
Liquid matching method		[19]
589	1.434	1.4338
Temperature matching method (47 °C)		[20]
589	1.4341	1.4336
Wavelength matching method		[19]
286	1.4566	1.4457
305	1.4534	1.4532
351	1.4464	1.4465
400	1.441	1.4412

as a function of the refractive index for calcium fluoride suspension is shown in Fig. 1. To find a location of the maximum transmittance (which defines the refractive index of the particle) one may use data-fitting procedures, which usually require at least a set of five different immersion liquids to get five data points, such as the Gauss-fitting procedure for the transmission T data as follows:

$$T = b + A \ \exp[-(n_{liquid} - n_{pigment})^2/(2\psi)^2], \quad (2)$$

where b is a baseline, A is an amplitude and ψ is related to the width of the normal distribution at the half maximum [18]. The refractive index estimate obtained by this data fitting method, which was applied to the data of Fig. 1, gave an estimate n_{589}=1.434. This result matches quite well with the literature value [19], as shown in Table 1. Careful examination of Fig. 1 reveals that the curve is not completely Gaussian shaped and thus there is a slight asymmetry. This phenomenon can be also observed when similar transmittance simulations are conducted using Mie-scattering calculus. A disadvantage of the traditional immersion liquid set method is that it is time-consuming and one has to usually utilize data fitting to get an estimate for the refractive index of the particle. There may be cases where the mathematical estimation of the turning point of the transmittance curve using Eq. (2) may give erroneous estimate especially if the number of immersion liquid samples is low. If the number is high better confidence is obtained but then the measurement time becomes longer. In the frame of the traditional immersion liquid set method quite often one has to deal with expensive and poisonous liquids that have to be correctly destroyed after the experiments. This is an issue of pollution of the environment. Furthermore immersion liquids are expensive.

Secondly, we determined the refractive index of CaF_2 by using the temperature method. The thermo-optic coefficients of CaF_2, glycerol and water were obtained from the literature and they are $-1.1\times10^{-5}\,°C^{-1}$ [20], $-2.4\times10^{-4}\,°C^{-1}$ [21] and $-9.9\times10^{-5}\,°C^{-1}$ [22], respectively. Based on literature data $n(t)$-curves can be plotted and such a plot for CaF_2 and glycerol–water mixture is shown in Fig. 2(a). We can observe from Fig. 2(a) that the refractive indices of CaF_2 and glycerol–water mixture are different except at the temperature of ca. 47 °C. To confirm this, a sample was prepared by weighing 4 g of CaF_2 and suspending it to the immersion liquid resulting in the total volume of 100 ml. The refractive index of this immersion liquid (glycerol 76 ml and water 24 ml), measured with the Atago refractometer, was 1.4395 at 589 nm and at room temperature 22 °C. The sample was heated in a temperature-controlled vessel and the temperature of the sample was observed using a thermocouple just before and after the transmission measurement. The wavelength of the spectrophotometer was set at 589 nm. Results are shown in Fig. 2(b) and in Table 1. After the interpolation the refractive index 1.4341 (589 nm) for CaF_2 at 47 °C was obtained. This is close to the literature value of 1.4335 (589 nm, 47 °C) [22]. Hence, a relatively good estimate for the refractive index of CaF_2 was obtained. Nevertheless, the sample is not thermally in equilibrium state because the cuvette and the sample compartment is not a closed, nor an isolated system. Thus there is heat exchange with the environment. This causes instability of the system and fluctuation of the data points thus the reliability of the measurement is an issue. The instability of the system is supported by the fluctuation of the data points in Fig. 2(b). One can calculate with the aid of the thermo-optic coefficients, given above, the change of the refractive index e.g. if the temperature changes. Obviously such a change of the temperature by a step of 1 °C in a sample causes a refractive index change of the order of 10^{-5}–10^{-4} for the substances of this study. The change is not a big issue when the system is in or near the thermal equilibrium i.e. the temperature of the sample is the same as that of the environments. When heating the sample, the temporal rate of heat exchange between the sample and reservoir is subjected to the fluctuations because of conduction and convection of heat. During the heating the temperature of the sample is more difficult to control too. Boundary layer convection will be present in the inner and outer envelope of the sample cuvette. Ambient disturbances like the ventilation of the room or moving of people can also affect the rate of the convection. Keeping the system at the desired temperature such as 47 °C is an issue because of the heat exchange between a small size cuvette and the surrounding big reservoir. If the temperature method is chosen a large volume of the sample is preferred because of the instability issue. Unfortunately, the measurement compartment of the spectrophotometer has usually its own restrictions regarding the size of the cuvette that can be used for measurement in the compartment where stray light of the environments should not be incident. Furthermore, thermo-optic coefficients of media are not constants but depend on the state of the thermodynamic system and are typically given only for pure substances. Hence, theoretical estimation of the refractive index at relatively high temperature can be an issue.

Finally, the wavelength method was examined. This method has been mentioned in various sources but as far as we know it has not been used especially in detection of the wavelength-dependent refractive index at various discrete wavelengths. The motivation of this study was to determine the refractive index of particle at several wavelengths because increasingthe amount of the refractive index data at various wavelengths also provides better confidence regarding the identification of the particle by its refractive index data. We exploited wavelength-dependent refractive index literature data of CaF_2 [20] and calculated the wavelength-dependent refractive index of

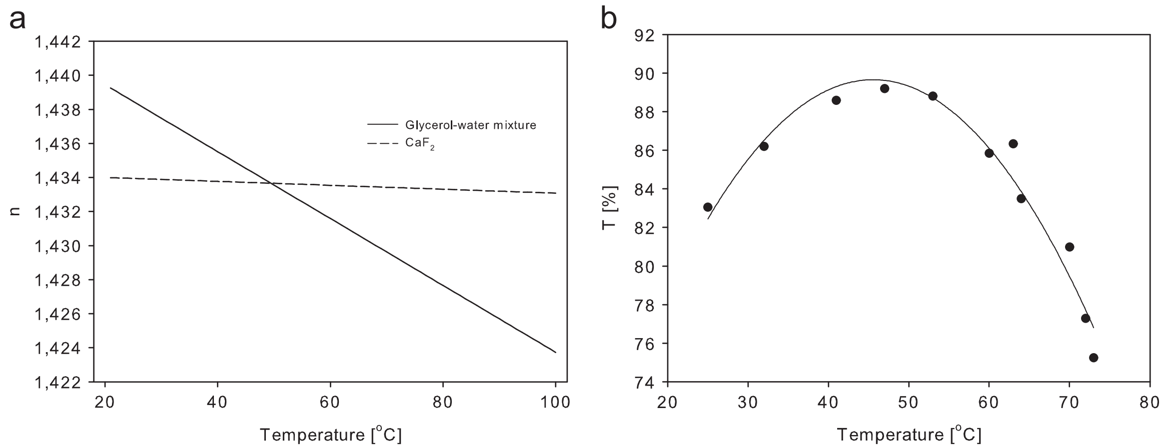

Fig. 2. The temperature method: (a) calculated refractive index, and (b) measured transmission as a function of temperature for CaF_2 and glycerol–water mixture at 589 nm. The solid line in (b) is used for fitting the experimental data.

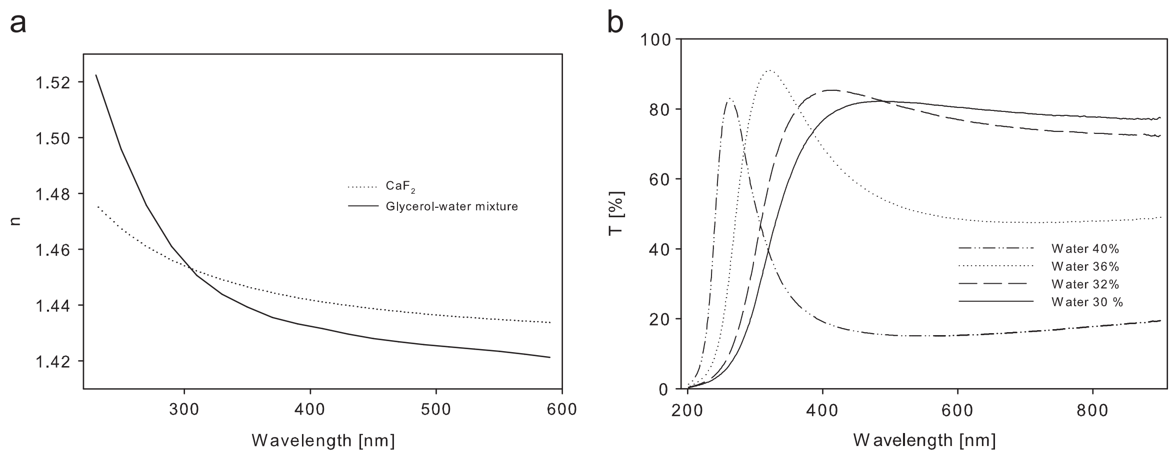

Fig. 3. The wavelength method: (a) calculated wavelength-dependent refractive indices of CaF_2 and a glycerol–water mixture, and (b) measured transmission data as a function of wavelength for different water/glycerol ratios (maximum transmissions representing index matching are located at 260 nm, 324 nm, 410 nm and 488 nm).

a glycerol water mixture using literature values for these substances [22,23]. By choosing one particular mixture we got one particular wavelength at which the refractive index of CaF_2 and water glycerol mixture is the same as shown in Fig. 3(a). It is evident from Fig. 3(a) that the refractive index of CaF_2 and that of glycerol water with mixture ratio (68%:32%) have different dispersion characteristics for all other wavelengths. If the mixture ratio of water and glycerol is chosen as a variable then one gets different wavelengths for the intersection point of the refractive index curves. This is an important observation if one wants to obtain refractive index at various discrete wavelengths i.e. to get information on light dispersion of the particle. The intersection of the refractive index curves is crucial because for that particular wavelength and that particular liquid mixture maximum light transmittance of the suspension is achieved. For such a purpose in this study we chose four glycerol–water mixtures (water/glycerol ratios: 30%, 32%, 36% and 40%) that gave transmission maximum at four different wavelengths. The refractive indices of these mixtures were measured with the Atago refractometer and were 1.4298, 1.4270, 1.4219 and 1.4159, at the wavelength of 589 nm and at 22 °C. In this case a lower total amount of CaF_2 than in the case of the two other measurements, which were explained above, namely 1 g was added to liquid mixtures (50 ml). Transmission curves from 200 to 900 nm were recorded with the spectrophotometer and the results are plotted in Fig. 3(b). The location of the maximum transmission represents the index matching condition and thus determines the refractive index of solid particles at four different wavelengths where the maximum transmittance is detected. It is obvious from Fig. 3(b) that sharper transmission peaks can be found at shorter wavelength range. In this case, the dispersion of CaF_2 and liquid almost equals in the visible wavelength range but differs at the UV-range, where the refractive index of the immersion liquid grows faster than that of CaF_2. Consequently, the light scattering increases rapidly (decreasing the transmittance) when the measurement wavelength has an off-set from the index matching condition. When the wavelength was scanned toward longer wavelengths from the maximum transmission, the light scattering increased at a slower pace due to similar dispersion of liquids and particles. Not only scattering but also particle size and concentration together with the observation wavelength have an effect on the sharpness of the transmittance curves shown in Fig. 3(b). In this study, the wavelength method does not provide refractive index value for CaF_2 at the wavelength of 589 nm. It is possible to get the refractive index at 589 nm if efforts are put to find more suitable liquid mixture. However, such a procedure was out of the scope of this study. Nevertheless, maximum transmissions could be found at the following wavelengths: 260, 324, 410 and 488 nm for the four liquid mixtures. Table 1 indicates that there is a relatively good agreement between the measurement results and literature values.

One issue is the sedimentation of the particles during the measurement. Sedimentation is a less serious problem in the case of the temperature method because warming of the liquid increases the Brownian motion of liquid molecules and also the solid particles. Shaking of the sample cuvette just before the measurement of the wavelength and immersion liquid set methods and quick measurement time (10 s in the case of the wavelength method) ensures reasonable homogeneity of the sample. The dynamic features of sedimentation, which was not a topic of this investigation, can also be studied by the wavelength method because one can choose a fixed wavelength for which there is a mismatch between the refractive index of the particle and the immersion liquid. Due to sedimentation the scattering of light is the strongest at the beginning of the measurement and it becomes weaker as a function of time. Hence monitoring of temporal changes of transmittance provides information about the dynamics of the sedimentation of microparticles. If the system is a colloid i.e. the particle size is of the order of nanometers then scattering and sedimentation are weak.

We estimate that the methods of this study can give at best the refractive index with the accuracy of 0.005 refractive index units which was obtained by calculating the average between the literature values and the results of the measurement. However, a pigment manufacturer reports typically refractive index of a particle with two-decimal accuracy. The refractive index difference between data given by the manufacturer and in literature can be explained by facts such as the origin and structure of the material and state of purity.

Main characteristics of different methods are listed in Table 2. All these measurements can be performed with a normal spectrophotometer that is often a basic optical apparatus in laboratories.

The conventional immersion liquid method is usually time-consuming if one has to prepare many mixtures of immersion liquids to cover a relatively wide refractive index range with small step of the refractive index. The challenge related to the temperature matching method concerns the precise temperature control of suspension. In addition, refractive index is determined at the measurement temperature making the comparison with the refractive index obtained at room temperature by other methods quite difficult. The wavelength matching method was the easiest and fastest (one transmittance measurement takes 10 s) way to determine the refractive index of calcium fluoride up to wavelength of 500 nm. Above 500 nm the dispersion of the used liquid and solid is similar in this case and the accurate determination of maximum point of transmission is a relatively difficult task. An advantage is that the analysis requires in practice a little volume (3 ml) of (sometimes expensive) immersion liquid compared to the two other methods. However, at the beginning for an unknown particle one has to find the suitable immersion liquids and their mixtures.

4. Conclusions

The immersion techniques are valuable tools to obtain information about the optical properties such as the refractive index and also mass concentration of particles. In this paper we have compared three liquid immersion techniques which can be used for the determination of the refractive index of tiny solid particles. Methods were tested using calcium fluoride particles and data obtained correlate well with the data found in the literature. The wavelength matching method was the easiest and fastest way among the three methods to determine the dispersion characteristics of the particle. In this study, where glycerol–water mixtures served as immersion liquids, the refractive index of CaF_2 was determined at discrete wavelengths in the spectral range of 200–500 nm. By carefully selecting other immersion liquids, with suitable optical dispersion, the usable spectral range can be extended to get the refractive index of CaF_2 (or other material) at other wavelengths too. Naturally, the magnitude of the refractive index, especially in case of an unknown particle, has a role regarding the choice of the liquid mixture. However, for a known particle which is a subject, for example, of a purity issue, the use of the liquid mixture is usually not a problem once suitable liquids have been found and tested for particles with expected optimal purity. Hence, a fast refractive index measurement can be based on the use of one *a priori* known refractive index of a liquid mixture and scanning of the incident wavelength. If higher confidence on the purity or identification of a particle is required then two or more different liquid mixtures can be used to collect dispersion data at various wavelengths. In another study, which will be published later, we have utilized the wavelength method of this article for the case of known contaminated particle suspension. The method was tested using CaF_2 particles with traces of different materials as impurities. Those results show that the monitoring of sub ‰-level contamination can be readily achieved.

Future trend, e.g., in pigment technology is that the optical properties of the particles can be tailored to suit the target applications by using the nanocoating or the nanodoping processes. The liquid immersion techniques can provide a useful tool to verify the refractive index of modified particles and thus to improve optical properties of paints, paper, cosmetic and pharmaceutical products. In nanomedicine where nanoparticles (NP) are used as drug carriers we suggest the wavelength method to find applications to identify different NPs from each other and to monitor the concentration of the NPs, e.g. in a fluid. Currently, liquid immersion methods are used for the determination of the

Table 2
Main characteristics of three liquid immersion methods.

Method	Pros	Cons
Liquid matching method	Broad (and narrow) refractive index sweep available No restriction concerning the wavelength	Two liquids needed Requires a relatively large amount of immersion liquid (can be expensive) Mixing procedure Time consuming. Applicable to one wavelength/measurements
Wavelength matching method	One immersion liquid needed mixture Fast and easy measurement Small liquid amount Suitable for purity inspection	Refractive index match typically occurs at a wavelength that one cannot predict or choose Dispersion characteristics of immersion liquid and particle have to be almost the same
Temperature matching method	One immersion liquid needed mixture	Results are not relevant to standard ambient conditions No data at room temperature Precise temperature control is challenging Long measurement time Liquids may chemically change and start to evaporate at high temperatures Knowledge of thermo-optical properties is needed.

refractive index of the particles in the laboratory. We think that the wavelength method could be a potential tool also for on-line applications in the pigment industries. It is possible to get information on the thermo-optical coefficient of liquids generally and also that of solid particles. Also development of the measuring geometry to study small volume of fluids in micro- and nano-fluidistics is a topic of future work in immersion liquid studies of the refractive index of micro- and nanoparticles.

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