The use of India ink in tissue-simulating phantoms

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Abstract: The optical properties of India ink, an absorber often used in preparation of tissue simulating phantoms, have been investigated at visible and near infrared wavelengths. The extinction coefficient has been obtained from measurements of collimated transmittance and from spectrophotometric measurements, the absorption coefficient from multidistance measurements of fluence rate in a diffusive infinite medium with small concentrations of added ink. Measurements have been carried out on samples of India ink from five different brands, and for some brands also from different batches. As also reported in previously published papers the results we have obtained showed large inter-brand and inter-batch variations for both the absorption and the extinction coefficient. On the contrary, our results showed small variations for the ratio between the absorption and the extinction coefficient. The albedo is therefore similar for all samples: The values averaged over all samples investigated were 0.161, 0.115, and 0.115 at λ = 632.8, 751, and 833 nm respectively, with maximum deviations of 0.044, 0.019, and 0.035. These results indicate that, using the values we have obtained for the albedo, it should be possible to obtain with uncertainty smaller than about 4% the absorption coefficient of a sample of unknown ink from simple measurements of extinction coefficient. A similar accuracy is not easily obtained with the complicated procedures necessary for measurements of absorption coefficient.

OCIS codes: (160.4760) Optical properties; (170.6510) Spectroscopy, tissue diagnostic; (170.7050) Turbid media.

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#135843 - $15.00 USD Received 28 Sep 2010; revised 11 Nov 2010; accepted 11 Nov 2010; published 7 Dec 2010

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1. Introduction

India ink is a readily available and inexpensive absorber widely used in preparation of tissue-simulating phantoms developed to check the performance of instrumentation for near infrared spectroscopy or imaging of tissues (see for instance [1, 2, 3, 4, 5, 6, 7, 8, 9]). It is added to suspensions of low absorbing diffusive media like polystyrene latex spheres, titanium dioxide, or lipid emulsions (e.g. Intralipid, Liposyn) to obtain the desired absorption. India ink is a particulate absorber: Absorption is due to insoluble carbon particles suspended in aqueous medium. With respect to molecular dyes, often used in particular to prepare liquid phantoms, India ink has many advantages and some disadvantages. The main advantages are that: it is chemically and spectroscopically stable, nontoxic, it does not fluoresce, and it is also suitable for preparing solid phantoms since, unlike dye molecules, ink particles cannot diffuse through a gel or polymer matrix. The main disadvantage is that unlike dyes which, being dispersed on molecular level, behave as pure absorbers, India ink, being a suspension, not only absorbs, but also scatters light. Its albedo is therefore not equal to zero and this makes difficult to measure the absorption coefficient, the most relevant optical property for the preparation of tissue phantoms. Furthermore, the absorption spectrum of India ink is smooth with relatively small variations over the visible and NIR region. For some applications this can be an advantage since it can be used for a large range of wavelengths, but for applications in which a phantom with large spectral variations in absorption is required this can be a disadvantage.

Although the optical properties of scattering media used for phantoms like lipid emulsions have been studied extensively [10, 11, 12], only few results are available for India ink. To our knowledge the deepest investigation on the optical properties of India ink for preparation of tissue phantoms has been reported by Madsen et al. [13]. They measured the extinction coef-

#135843 - $15.00 USD Received 28 Sep 2010; revised 11 Nov 2010; accepted 11 Nov 2010; published 7 Dec 2010

ficient, the single scattering albedo and the asymmetry factor for samples from two different brands of ink (Higgins and Regal) and also from different batches (two batches of Regal). The extinction coefficient was obtained from measurements of collimated transmittance, the albedo and the asymmetry factor from measurements of diffuse reflectance and transmittance using standard integrating sphere techniques. All measurements were carried out at λ = 594 nm. The size distribution of suspended particles was also investigated using an electron microscope. The results reported in [13] showed that 1) the suspended particles have an approximately bimodal size distribution with diameters around 0.1 and 1.0 μm (in a typical micrograph there were approximately 200 small and 3 ± 1 large particles), 2) India ink has a significant scattering component (the values for the albedo ranged between 0.29 and 0.43), 3) there are large inter-brand and inter-batch variations both for the extinction coefficient and for the albedo, and 4) the optical properties of a given ink solution appear to be stable over a period of several months. Based on these results Madsen et al. concluded that the determination of the optical properties of India ink is a tedious procedure that must be repeated for every sample of ink, and that a simpler alternative is to use, where possible, non-particulate absorber such as molecular dyes, for which, being scattering insignificant, absorption can be simply determined from measurements of collimated transmittance or from spectrophotometric measurements.

The values of albedo reported by Madsen et al. [13] are significantly larger with respect to values reported in other papers. From measurements of collimated transmittance, of diffuse reflectance and transmittance with the integrating sphere Royston et al. [14] obtained the value 0.12 ± 0.06. They carried out measurements at λ = 1064 nm on samples of Pelikan India ink. Xu and Patterson [15] obtained the extinction coefficient with a spectrophotometer and the absorption coefficient from measurements of effective attenuation coefficient carried out on a dilution of Intralipid with different ink concentrations. From the results they reported at λ = 750 nm for a sample of Demco India ink a value close to zero for the albedo (0.06) is obtained. Results at 750 nm have been also reported by Martelli and Zaccanti [16] for a sample of Rotring India ink. The extinction coefficient was obtained from measurements of collimated transmittance, and the absorption coefficient from measurements of effective attenuation coefficient carried out for different ink concentrations on a dilution of previously calibrated Intralipid. The value obtained for the albedo was 0.125 ± 0.020. The absorption coefficient of the same sample of Rotring ink was also measured using a completely different method based on time resolved measurements by Spinelli et al. [17]. The results obtained with the two methodologies were almost identical (difference smaller than 1%). Finally, in other papers [5, 9] the absorption coefficient of India ink has been measured with a spectrophotometer making the assumption that scattering is negligible with respect to absorption, i.e., assuming the albedo equals to zero.

Before being used for tissue phantoms, the optical properties of suspensions of India ink had been studied in view of their use as sunlight absorber fluids in solar collectors. Wagner et al. [18] measured the extinction and the absorption coefficients of Higgins ink in the 370 nm to 720 nm range. They observed that the extinction and the absorption coefficients decrease slightly with increasing wavelength, but the albedo was approximately independent of wavelength with a value around 0.15.

The values reported in the literature show large differences in the optical properties of India ink measured by different laboratories. These differences can be explained with inter-brand and inter-batch variations as shown in the Madsen et al. paper [13], with different procedures for preparing dilutions, and also with different wavelengths used for the experiments, but in our opinion the suspicion remains that at least in part the differences observed, especially for the single scattering albedo, may be ascribed to the different methods used in different laboratories to measure the optical properties: As pointed out by Madsen et al. [13] and by Xu and Patterson [15], the determination of the optical properties of India ink is a tedious procedure and a non

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trivial task. A further investigation on the optical properties of India ink at visible and near infrared wavelengths is therefore desirable.

In this paper we report the results of measurements of absorption and extinction coefficient for nine samples of India ink at λ = 632.8, 751, and 833 nm. Measurements have been carried out on samples of India ink from five different brands, and for some brands also on samples from different batches. For some samples the spectrum of the extinction coefficient between 400 and 1000 nm has been also measured. The results we have obtained confirm the large inter-brand and inter-batch variations for both the absorption and the extinction coefficient but, on the contrary, small variations have been observed for the ratio between the absorption and the extinction coefficient, showing that the albedo is very similar for all samples we investigated. Furthermore, in spite of significant variations of the absorption and extinction coefficients with wavelength, small variations have been observed for the albedo especially at NIR wavelengths. These results indicate that, using the values we got for the albedo, it should be possible to obtain with a good accuracy the absorption coefficient of a sample of ink from simple measurements of the extinction coefficient without the need for the complicated procedures necessary for the direct measurement.

2. Materials and methods

We have carried out measurements on the nine samples of India ink (seven bottles and two cartridges) shown in Fig. 1. We used samples from five different brands (Higgins, Rotring, Pelikan, Staedtler, and Koh I Noor) and also from different batches (three batches of Pelikan, two of Higgins, and two of Rotring). We have measured the extinction coefficient μ_e and the

Fig. 1. India ink used for measurements. Samples are from five different brands (Higgins, Rotring, Pelikan, Staedtler, and Koh I Noor) and for some brands also from different batches (three batches of Pelikan, two of Higgins, and two of Rotring).

absorption coefficient μ_a of dilutions in purified water (Elix 3 Millipore purification system) from which we obtained the specific extinction and absorption coefficients $\varepsilon_{e\,ink}$ and $\varepsilon_{a\,ink}$ of non diluted ink, i.e., the coefficients of proportionality relating μ_e and μ_a to the weight concentration ρ_{ink} of ink

$$\mu_e(\rho_{ink}) = \varepsilon_{e\,ink}\rho_{ink} \quad (1)$$

$$\mu_a(\rho_{ink}) = \varepsilon_{a\,ink}\rho_{ink}. \quad (2)$$

#135843 - $15.00 USD Received 28 Sep 2010; revised 11 Nov 2010; accepted 11 Nov 2010; published 7 Dec 2010

Measurements have been carried out at three wavelengths: λ = 632.8, 751, and 833 nm. We have used a He-Ne laser and two laser diodes. The specific extinction coefficient has been obtained from measurements of collimated transmittance as a function of the ink concentration using an experimental setup similar to that described in [19]. The acceptance angle of the detection system was of 7 mrad. With this acceptance angle the error on the extinction coefficient due to the unavoidable fraction of scattered received power remains small (less than 0.2%) even assuming for the scattering function of ink particles in the forward direction a value as high as 100 [19, 20].

To measure the absorption coefficient we have used the method described in [16] based on measurements of effective attenuation coefficient μ_{eff} as a function of the concentration of India ink carried out on a diffusive medium with known reduced scattering coefficient. If the added ink does not significantly alter the reduced scattering coefficient of the diffusive medium, μ_{eff} is related to ρ_{ink} by

$$\mu_{eff}^2(\rho_{ink}) = 3\mu_{s0}^{'}(\mu_{a0} + \varepsilon_{a\,ink}\rho_{ink}) \tag{3}$$

where μ_{a0} and $\mu_{s0}^{'}$ are the absorption and the reduced scattering coefficient of the diffusive medium before the addition of ink. The specific absorption coefficient is obtained as

$$\varepsilon_{a\,ink} = \frac{S_{ink}}{3\mu_{s0}^{'}} \tag{4}$$

where S_{ink} is the slope of the straight line that best fits $\mu_{eff}^2(\rho_{ink})$ as a function of ρ_{ink}. The assumption that the added absorber does not significantly alter the reduced scattering coefficient is well verified in our experiments since we added few milliliters of previously diluted ink to $\simeq$ 3L of diffusive medium.

The effective attenuation coefficient has been obtained from multidistance measurements of fluence rate inside an infinite medium illuminated by a CW source. The diffusive medium we have used is a suspension of Intralipid 20% whose reduced scattering coefficient was previously calibrated with an uncertainty less than 2%. At λ = 632.8 nm Intralipid has been calibrated using the method of adding absorption [19], at 751 and 833 nm with the method of absorption of water [16]. For measurements of effective attenuation coefficient we have used suspensions with $\mu_s^{'} \cong 1.4$ mm^{-1} at 632.8 nm, and $\mu_s^{'} \cong 0.7$ mm^{-1} at 751 and 833 nm. For these values of $\mu_s^{'}$ the volume of diffusive medium we used, a cylinder with volume of about 3 liters, is sufficiently large to act as an infinite medium.

From $\varepsilon_{e\,ink}$ and $\varepsilon_{a\,ink}$ the single scattering albedo of India ink has been evaluated as $\Lambda_{ink} = (\varepsilon_{e\,ink} - \varepsilon_{a\,ink})/\varepsilon_{e\,ink}$.

The experimental setup we have used is the same described in [12]. For multidistance measurements two thin fibers with a small diffusive tip (outer diameter 0.5 mm) having a substantially uniform radiation pattern (US Patent Number 6,071,302 'Phototherapeutic apparatus for wide-angle diffusion') were used to illuminate the medium and to measure the fluence. The interfibre distance was varied between 20 and 45 mm (1 mm step) with a computer controlled translation stage and received photons were measured with a photomultiplier and a lock-in amplifier. For collimated transmittance measurements we have used a scattering cell 34.5 mm thick, and transmittance was measured with a photodiode and a lock-in amplifier.

We point out that measurements of optical properties for comparisons among the different brands and the different batches of India ink, have been carried out in a short time (few days), and for each wavelength measurements have been carried out, as far as possible, with identical experimental conditions: We have used the same experimental setup and dilutions with very similar concentrations. From a measurement to the other, we only changed the sample to be investigated, so that systematic errors are almost identical. This is particularly important for

#135843 - $15.00 USD Received 28 Sep 2010; revised 11 Nov 2010; accepted 11 Nov 2010; published 7 Dec 2010

measurements of ε_{aink} in which systematic errors, due to the error on the calibration of the diffusive medium, to the finite volume of the medium, and to the uncertainty in positioning the fibers, are higher than random errors in the measured fluence and concentration.

For four samples of ink (Higgins A, Higgins B, Rotring A, and Pelikan A) we have also measured the spectrum of the extinction coefficient between 400 and 1000 nm using a Perkin Elmer Lambda 19 spectrophotometer.

Since the absorption coefficient of India ink is very high (hundreds of mm^{-1}), a very small quantity is sufficient to obtain the values of the absorption of interest for tissue optics (of the order of one hundredth of mm^{-1}). Pre-diluted ink is therefore commonly used to obtain the concentration with good accuracy. In our measurements the weight concentration has been determined with an error smaller than 0.3%. To monitor temporal variations of the optical properties of diluted ink, measurements of collimated transmittance have been repeated using the same diluted sample over a period of about one year. The diluted samples were stored in plastic or glass bottles (100 ml to 500 ml) in the laboratory at room temperature (between 10°C and 30°C). During ultrasound application the temperature increased to about 40°C.

In our experience it is very important to apply ultrasound both before preparing the pre-dilution of India ink and before using it: Before using, we immerse the bottle of ink or of diluted ink in an ultrasonic water bath for about 30 minutes. Without ultrasound application, we have obtained significantly different values for extinction and absorption coefficient even for identical dilutions prepared from the same bottle.

3. Experimental results

As mentioned in Section 2 we expect that both the extinction and the absorption coefficient of diluted ink linearly increase with the ink concentration. To check the linearity, Fig. 2 shows an example of results for μ_e and μ_a as a function of the ink concentration. The extinction coefficient has been obtained from measurements of collimated transmittance and actually represents the extinction due to ink. The absorption coefficient has been obtained from measurements of effective attenuation coefficient dividing μ_{eff}^2 by $3\mu_{s0}^{'}$ (Eq. (3)). It therefore includes both the absorption due to ink and the absorption due to the diffusive medium. The error bars on the results both for extinction and for absorption coefficient are smaller than the marks. The straight line that best fits the results is also shown both for the extinction and for the absorption coefficient. The range of ink concentrations for which results have been reported is limited by the uncertainty of the measurement. However, the corresponding values of extinction and absorption coefficient probably cover the range of values of practical interest for tissue optics. These results pertain to the sample of Higgins A at λ = 632.8 nm.

The results obtained from measurements on the nine samples from different brands and different batches of India ink are summarized in Fig. 3. The figure shows the results for the specific extinction and absorption coefficients ε_{eink} and ε_{aink} at λ = 632.8, 751, and 833 nm, together with the corresponding results for the ratio $\varepsilon_{aink}/\varepsilon_{eink}$. The error bars for ε_{eink} and ε_{aink} have not been reported since smaller than the marks. Those for $\varepsilon_{aink}/\varepsilon_{eink}$ represent the standard error due to random errors (the systematic error, smaller than 2%, due to the uncertainty on the calibration of the reduced scattering coefficient of the diffusive medium has not been included). The values averaged on the nine samples are also shown for the ratio $\varepsilon_{aink}/\varepsilon_{eink}$ (horizontal lines). At 751 and 833 nm the average values are very close, and the corresponding lines are indistinguishable. Both for ε_{eink} and ε_{aink} there are strong variations not only for samples of different brands, but also for samples from different batches of the same brand. On the contrary, almost indistinguishable results have been obtained for different samples from the same batch. However, in spite of the large variations observed for ε_{eink} and ε_{aink} (for each of the three wavelengths the ratio between the maximum and the minimum value was about 2.5)

#135843 - $15.00 USD Received 28 Sep 2010; revised 11 Nov 2010; accepted 11 Nov 2010; published 7 Dec 2010

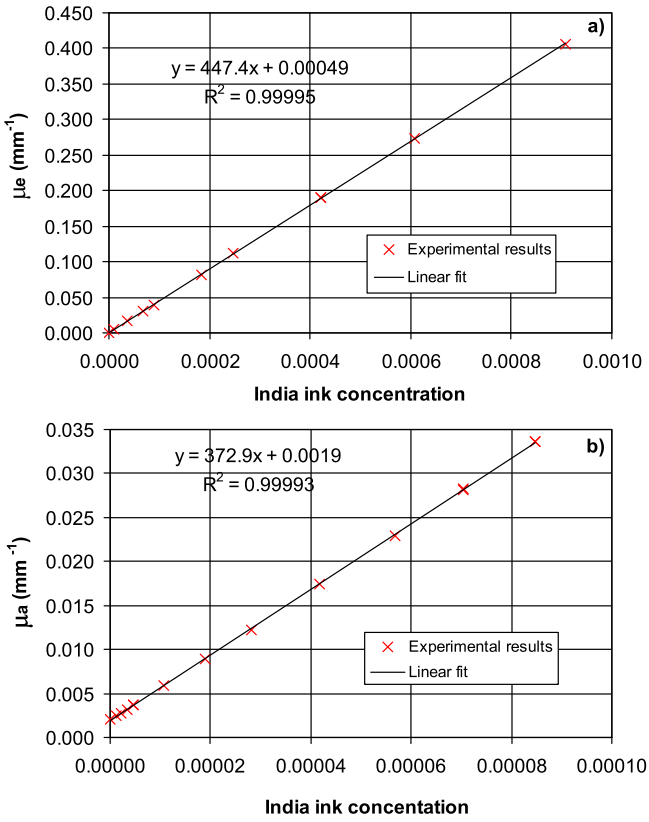

Fig. 2. Extinction and absorption coefficient as a function of the India ink concentration. The results pertain to the sample of Higgins A at λ = 632.8 nm.

small batch-to-batch and brand-to-brand variations have been observed for the ratio $\varepsilon_{a\,ink}/\varepsilon_{e\,ink}$: The values averaged over the nine samples are 0.839, 0.885, and 0.885 at λ = 632.8, 751, and 833 nm respectively, and the corresponding maximum deviations from the average are 5.2%, 2.2%, and 3.9%. For the single scattering albedo the average values at the three wavelengths are 0.161, 0.115, and 0.115 with maximum deviations of 0.044, 0.019, and 0.035. Being the albedo close to zero the corresponding maximum percentage deviations are large (up to 30%), but for practical applications these deviations can be considered reasonably small if we assume the absorption coefficient to be evaluated from measurements of the extinction coefficient with these values for the albedo. In fact, starting from accurate measurements of the extinction coefficient the absorption coefficient can be obtained with uncertainty smaller than about 4%. A similar accuracy is not easily obtained in measurements of the absorption coefficient.

The results of spectrophotometric measurements are shown in Fig. 4. Since it is particularly interesting to compare the shape of the spectra (the absolute values at λ = 632.8, 751, and 833 nm are shown in Fig. 3a)) each spectrum has been normalized to its value at 700 nm. The shapes of the four spectra are very similar. In particular, the curves for Higgins A, Higgins B, and Pelikan A are almost indistinguishable. The results of spectrophotometric measurements at λ = 633, 751, and 833 nm were in good agreement with collimated transmittance measurements: Discrepancies were within the standard errors (0.5% for collimated transmittance measure-

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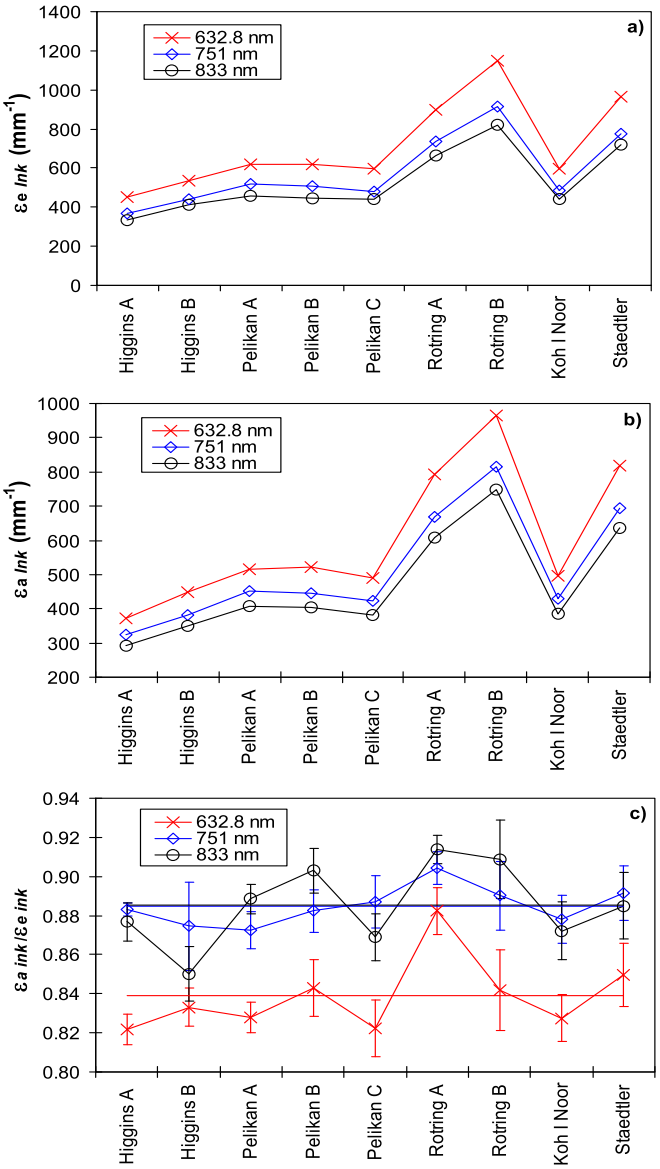

Fig. 3. Summary of the results obtained from measurements on nine samples of India ink from different brands and different batches. The results for the specific extinction and absorption coefficients $\varepsilon_{e\,ink}$ and $\varepsilon_{a\,ink}$ are reported together with the results for the ratio $\varepsilon_{a\,ink}/\varepsilon_{e\,ink}$.

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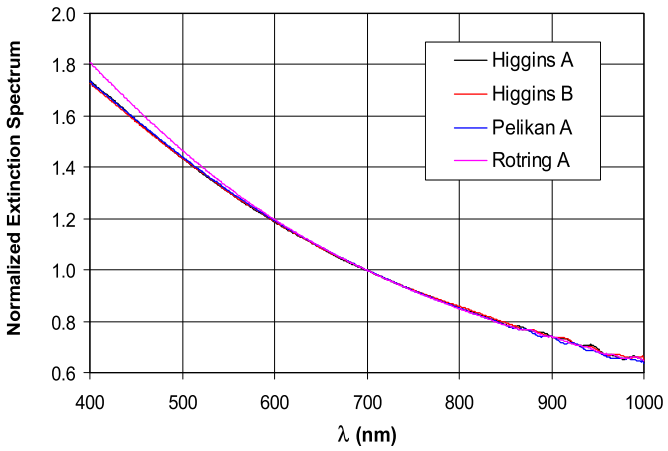

Fig. 4. Comparison among the normalized spectra of extinction coefficient for three brands of India ink. The curves for Higgins A, Higgins B, and Pelikan A are almost indistinguishable.

ments and 2% for spectrophotometric measurements).

As mentioned above, due to the high absorption of India ink, pre-diluted ink is commonly used to prepare concentrations of practical interest. It may be therefore interesting to know how long the optical properties of diluted ink remain stable. For this purpose the extinction coefficient of a dilution of Higgins A ink has been monitored over a period of about one year. The results at the three wavelengths are shown in Fig. 5. For each wavelength the figure also

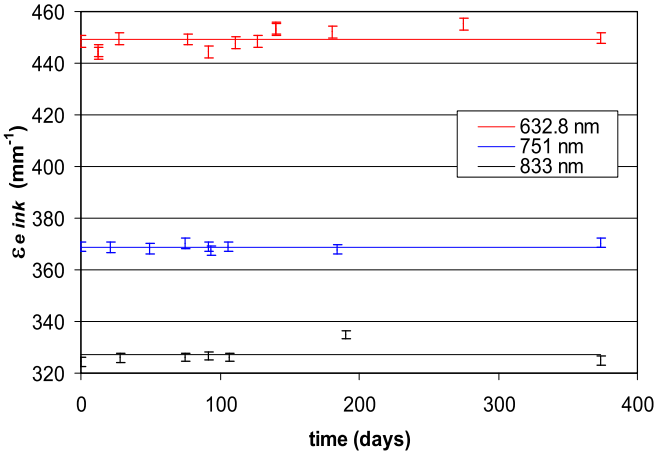

Fig. 5. Results of measurements of extinction coefficient carried out on the same dilution of ink (Higgins A) over a period of about one year. The horizontal lines represent the average values at the three wavelengths.

shows the average value (horizontal lines). Variations remain within about 1% (comparable with the standard error) apart from measurements carried out after 190 days at 833 nm and after 275 days at 632.8 nm, for which variations were of 2.4% and 1.3% respectively. Also referring to

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other measurements on different dilutions, we ascribed these slightly larger variations to a too short ultrasound application.

4. Discussion and conclusions

The results of measurements carried out on nine samples of India ink from different brands and different batches showed that:

1) Samples from different brands or from different batches of the same brand can have significantly different values for $\varepsilon_{e\,ink}$ and $\varepsilon_{a\,ink}$. On the contrary, samples from the same batch have almost identical optical properties;

2) In spite of the large brand-to-brand and batch-to-batch variations observed for the specific extinction and absorption coefficients, very similar values have been obtained for the ratio $\varepsilon_{a\,ink}/\varepsilon_{e\,ink}$ and for the single scattering albedo;

3) The spectrum of the extinction coefficient is similar for the four samples we measured.

4) The optical properties of diluted ink remain stable for a long time. The extinction coefficient of a diluted sample of Higgins A ink has been monitored for about one year without observing significant variations;

5) As expected, the extinction and the absorption coefficient of diluted ink is proportional to the ink concentration for dilutions of practical interest for tissue phantoms;

6) India ink can be added to Intralipid without changing its scattering properties.

The most significant of the results we have obtained is probably that for the ratio $\varepsilon_{a\,ink}/\varepsilon_{e\,ink}$ and for the albedo, that slightly depend on the brand and on the batch of India ink. Furthermore, variations with wavelength are small especially in the near infrared (the average values at 751 and 833 nm are almost identical).

This result suggests that measurements of the extinction coefficient, together with the average values of $\varepsilon_{a\,ink}/\varepsilon_{e\,ink}$ reported in Section 3, can be used to obtain the absorption coefficient with an uncertainty of less than about 4%. We point out that accurate values for the extinction coefficient are easily obtainable from measurements of collimated transmittance or from spectrophotometric measurements; on the contrary, as mentioned in Section 1, measurements of the absorption coefficient are rather complicated and it may be difficult to obtain accurate results.

Figure 4 shows a smooth dependence of the extinction coefficient on wavelength. The spectra are well fitted by a power law λ^b. From the fit of the curves of Fig. 4 we obtained for the coefficient b averaged over the four samples and for the corresponding standard deviation $b = -1.12 \pm 0.02$. A similar value, $b = -1.11 \pm 0.07$, has been obtained from the fit of measurements at λ = 632.8, 751, and 833 nm on the nine samples.

Also the results for the absorption and the scattering coefficients at the three wavelengths have been fitted with a power law. Averaging the coefficient b over the nine samples we obtained $b = -0.90 \pm 0.04$ for the absorption and $b = -2.43 \pm 0.50$ for the scattering coefficient. These values suggest that the size of carbonaceous particles of India ink should be small with respect to the wavelength, although they are different from those for Rayleigh scatterers. In fact, if the refractive index (real and imaginary part) is independent of λ, for Rayleigh scatterers the dependence is λ^{-1} for the absorption coefficient and λ^{-4} for the scattering coefficient [21].

The very similar results we obtained for the albedo and for the shape of the spectrum of the extinction coefficient of different samples also suggest that the different brands and the different batches of India ink we investigated should have similar shape and size distribution for the suspended particles and similar refractive index (real and imaginary part). A reasonable explanation of the results obtained is therefore that carbon particles in different brands have similar optical properties, and that observed brand-to-brand and sample-to-sample differences in the extinction coefficient are mainly due to different particle concentrations.

Comparison with previously published results show that the results we have obtained for the

#135843 - $15.00 USD Received 28 Sep 2010; revised 11 Nov 2010; accepted 11 Nov 2010; published 7 Dec 2010

albedo are similar to the values reported in [14, 16, 17, 18], and slightly larger with respect to the value in [15]. As for the comparison with the results reported by Madsen et al. [13], we too observed the large inter-brand and inter-batch variations for the extinction and the absorption coefficient, and the stability of a given ink solution over a period of several months, but for the albedo our results are significantly different and also the spectra of the extinction coefficient seem to be in contrast with the size distribution reported in [13]. For the albedo, differently from Madsen et al., we observed small inter-brand and inter-batch variations and also the values were significantly different: Madsen et al. reported values between 0.29 and 0.43 at 594 nm; our results at 632.8 nm are between 0.12 and 0.18. For the size distribution of ink particles we have no direct observations, however some information can be obtained from the spectrum of the extinction coefficient and from the albedo. For this purpose we used Mie theory to simulate the optical properties of carbonaceous spheres of different size suspended in water. Results are reported in Fig. 6. The figure shows the normalized spectrum for the extinction coefficient and

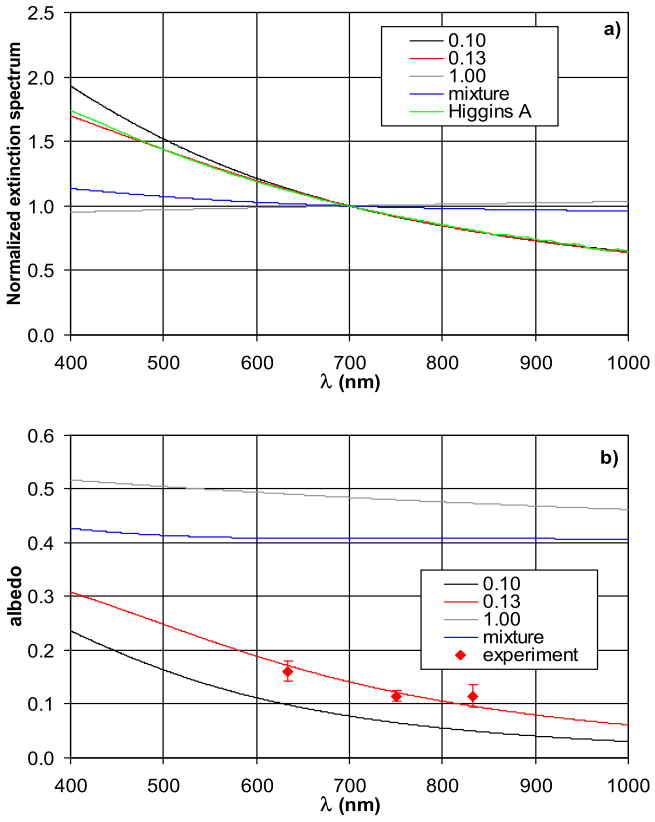

Fig. 6. Comparisons with Mie theory: The normalized spectrum for the extinction coefficient measured for the Higgins A sample and the values for the albedo averaged over the nine samples are compared with Mie theory simulations for spheres of different diameter (0.1, 0.13, and 1 μm) and for the bimodal distribution (mixture) of large (1 μm) and small (0.1 μm) particles reported in [13].

the spectrum for the single scattering albedo for particles with diameter 0.1, 0.13, and 1 μm and for a mixture with the bimodal distribution observed by Madsen et al., with 3 large particles (1 μm) every 200 small particles (0.1 μm). The optical properties of the mixture are dominated

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by the few large particles (they occupy a volume 15 times larger than small particles) and show a slight dependence on wavelength. The results for the spectra are compared with the spectrum measured for the Higgins A sample, those for the albedo with the values averaged over the nine samples. The experimental results are close to those for 0.13 μm particles, but significantly different from those for the mixture.

For Mie theory simulations we used for the real and imaginary part of the refractive index the values 1.95 and 0.79 (independent of the wavelength) suggested in the investigative review of Bond and Bergstrom [22] for light absorbing carbonaceous particles suspended in the atmosphere. Very similar results have been obtained using the values 1.96 and 0.66 [23] also used by Madsen et al. for simulations at 594 nm. We point out that comparisons with Mie theory should be used with caution. In fact, results similar to those from experiments can be also obtained using different size distributions of the suspended particles. Furthermore, if considerations reported by Bond and Bergstrom on the structure of carbonaceous particles are applicable to India ink, scattering and absorption of these particles are not like the ones of spherical particles and Mie theory is not applicable.

A possible explanation of the discrepancies observed between our results and those obtained by Madsen et al. [13] could be the ultrasound application that reduces the number of large particles (as suggested by Madsen et al. large particles may partly consist of aggregates of smaller particles). However, as mentioned before, measurements on samples without ultrasound application showed significant variations for extinction and absorption, but we have never observed significantly different results for the albedo although we must point out that a systematic investigation on the albedo of these samples has not been done.

We stress again that in our experience to obtain samples with reproducible optical properties the ultrasound application is very important both before preparing the pre-dilution of India ink and before using it. Furthermore, in using pre-diluted ink it is also important to keep the bottle with the dilution tightly closed to avoid changes due to evaporation of water.

Acknowledgements

The research leading to these results has received funding from the European Community's Seventh Framework Programme [FP7/2007-2013] under grant agreement n FP7-HEALTH-F5-2008-201076. The authors thank the colleagues of Dipartimento di Fisica-Politecnico di Milano and Marcello Picollo of IFAC-CNR di Firenze for kind help with spectrophotometric measurements, Maddalena Patrini and Rosalba Saija for suggestions on the refractive index of carbon particles, and Danilo Marcucci for help during the experiments.

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