Non-destructive measurements of the optical properties of apples by means of time-resolved reflectance

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Keywords

chlorophyll, absorption, scattering, photon migration, fruit

Abstract

Time-resolved reflectance is proposed and effectively used for the non-destructive measurement of the optical properties in apples. The technique is based on the detection of the temporal dispersion of a short laser pulse injected into the probed medium. The time-distribution of re-emitted photons interpreted with a solution of the Diffusion equation yields the mean values of the absorption and reduced scattering coefficients of the medium. The proposed technique proved valuable for the measurement of the absorption and scattering spectra of different varieties of apples. No major variations were observed in the experimental data when the fruit was peeled, proving that the measured optical properties are referred to the pulp. The depth of probed volume was determined to be about 2 cm. Finally, the technique proved capable to follow the change in chlorophyll absorption during storage.

Introduction

The assessment of the internal quality of fruits is getting a crucial task in an ever more competing market. Further, there is an increasing demand for non-destructive and non-invasive tests to determine the internal quality of every single fruit or to follow-up the storage process or even to determine the best pick-up time for harvesting.

Different non-destructive techniques have been proposed to probe different quality-related factors in fruits[1]. For example, interesting results have been shown with the use of ultrasound techniques for the assessment of defects in cherries[2]. Also, photoacustic techniques have proven useful for the detection of anthocyanins in strawberries[3]. The idea of the artificial nose, with the possibility to detect small quantities of released chemicals is particularly challenging[4] even though few data are available yet on its possible use. On the other hand, NMR is appealing in terms of specificity and spatial resolution[5] but is not adequate for on-the-field or mass applications.

Another promising approach implies the use of visible or near-infrared light. Different techniques have been already devised and effectively used based on the measurement of the total diffusely reflected light at different wavelengths. For instance, in the visible region of the spectrum, colorimetry has been used to determine the color of the skin[6]. Also, in the near infrared region, the spectrum of re-emitted light has been studied mainly to estimate the total sugar content[7].

A key limitation of these techniques is due to the fact that the intensity of the diffusely remitted light is strongly dependent on the color of the skin that hides the information from the pulp. Moreover, the total reflected intensity is determined both by the absorption and the scattering properties, making it unfeasible to separate the effects of the two parameters. Absorption and scattering contain distinct information on the medium. Absorption is determined by the pigments and constituents of the pulp that yields characteristic spectral features in the visible and near infrared region of the spectrum. Conversely, scattering is due to the local variation of the dielectric constant inside the medium. Microscopic changes in refractive index caused by membranes, air vacuoles, or organelles deviate the photon paths and are ultimately responsible for light diffusion. Macroscopically, this phenomenon can be described using the scattering coefficient (μ_s) that corresponds to the mean distance between interaction sites, and the angular probability distribution of scattered photons. A further simplification assumes a single parameter, the transport or reduced scattering coefficient $\mu'_s = \mu_s \cdot (1-g)$, where g is the mean cosine of the scattering angle for a single scattering event. This parameter corresponds to the effective scattering coefficient assuming isotropic scattering, and is adequate to describe light distribution for highly scattering media (e.g. apples)[8].

In this paper we show, for the first time to our knowledge, the use of a Time-Resolved Reflectance technique (TRR) for the non-invasive measurement of both the scattering and the absorption properties of turbid fruits. This technique is based on the measurement of the temporal delay and broadening experienced by a short laser pulse while traveling through a turbid medium[9]. Usually, the laser light is injected to and collected from the medium using a couple of fibers placed in contact with the surface at a fixed relative distance. If a proper theoretical model is used for the analysis of the experimental data, it is possible to measure both the absorption coefficient (μ_a) and the reduced scattering coefficient of the probed medium with good accuracy[10,11].

This technique has been already successfully used for the detection of optical properties in biological media for biomedical applications[12]. Here we propose the use of TRR to measure the absorption and scattering spectra of fruits and vegetables in the wavelength range from 610 nm to 700 nm. The influence of the skin in the TRR measurement is studied, as well as the depth of the volume probed by the technique. Finally, an exemplum on the changes of optical properties due to aging is shown.

Materials and Methods

1. Instrumentation

A synchronously pumped mode-locked dye laser is used as the illuminating source. The dye (DCM) is pumped by an actively mode-locked Argon laser (CR-18, Coherent, CA). The repetition rate of the pulses is reduced down to about 9 MHz by means of an intracavity cavity dumper. The dye laser is tunable between 610 nm and 700 nm, with an average power of about 10 mW and a pulse width < 20 ps. The laser light is injected to, and collected from the sample by means of 1 mm core 1 m long plastic-glass fibers set on the fruit surface at a relative distance of 1.5 cm. A proper fiber holder keeps the fibers in contact with the sample one parallel to the other and avoids direct collection of specular reflected light. The distal end of the collecting fiber is placed at the entrance slit of a scanning monochromator (HR-250, Jobin Yvon, France), coupled to a double microchannel plate photomultiplier (R1564U, Hamamatsu, Japan). Then, the signal is processed by an electronic chain for time-correlated single-photon counting. The triggering signal is provided by coupling part of the laser light to a fast photodiode. A small fraction of the incident beam is also coupled to another fiber and directly fed to the entrance slit of the monochromator to compensate for any temporal drift of the electronic chain. The temporal width of the instrumental transfer function is < 120 ps (FWHM) as measured facing directly the injection and collection fibers. All the system is controlled by a PC that allows one to automatically acquire a set of time-resolved reflectance curves over a given wavelength range. Typically, the overall time required to acquire a set of time-resolved reflectance measurements from 610 nm to 700 nm every 5 nm with 100,000 counts per curve is about 1 min.

2. Analysis

The temporal profile of the TRR curve is analyzed using a solution of the Transport Equation under the Diffusion approximation for a semi-infinite homogeneous medium[10] that takes into account the refraction index mismatch at the surface[13]. The experimental curve is fitted with a convolution of the theoretical function with the instrumental transfer function. The best fit is reached minimizing the χ^2 varying both μ_a, and μ'_s using a Levenberg Marquard iterative procedure[14]. The range of the fit includes all the points of the experimental curve with a number of counts higher than 80% of the peak value on the raising edge of the curve and 1% of the peak value on the falling edge. Figure 1 shows the best fit of a typical experimental curve. The instrumental transfer function is also shown for comparison (dashed line). The fitting procedure can automatically analyze a full batch of experimental curves on a standard Pentium PC at a speed of 10 curves per second. Synchronization of the analysis PC with the measurement one over the network permits on-line processing of the experimental

data, so that the absorption and scattering spectra are shown on the screen in real-time while the measurement is in progress.

Results

The accuracy of a TRR measurement for the detection of μ_a and μ'_s on a turbid medium has been already discussed elsewhere[15,16]. Generally speaking, for a given set of μ_a and μ'_s values, the accuracy of the measurement increases for higher values of the interfiber distance provided that enough photons can be collected (about 100,000). We have shown that, for an interfiber distance $\rho = 1.4$ cm, $\mu'_s = 10$ cm^{-1}, and $\mu_a < 1$ cm^{-1}, the measurement of μ_a is accurate within 10%, while the measurement of μ'_s within 20%[14]. For $\rho = 1.5$ cm as used in the present study, and a μ'_s value around 10 cm^{-1} or higher we expect a comparable or even better accuracy.

The TRR technique has been applied for the first time to our knowledge to the non-invasive measurement of μ_a and μ'_s spectra in Fruits. Figure 2 shows the absorption (left pane) and scattering (right pane) spectra obtained on intact apples. Three different varieties were selected that is Golden (top row) Granny-Smith (middle row) and Starking apples (bottom row). For each variety, 2 different fruits were measured (diamonds and triangles) and, for each of them, 2 sides were probed. The idea was to measure the front side more exposed to sunlight (filled symbol) and the back side (open symbols). Clearly this selection was not always evident – as in the case of Granny-Smith – yet the criterion was to check the largest variation in optical properties observed in the very same fruit. Looking at the absorption spectra, the key spectral feature is the peak around 675 nm, that is easily attributed to chlorophyll (CHL). Great variations are found among different varieties, being Granny-Smith normally richer of CHL, and among different fruits of the same variety. Also, changes are observed on the same fruit probing different sides, even though the internal variation is lower then the inter-fruits variability. In apples with quite a low CHL absorption (e.g. Golden, triangles), the absorption spectrum is characterized by a slightly decreasing plateau, that is probably the tail of anthocyanine spectra peaked in the blue. Water absorption is negligible in this wavelength range (<0.005 cm^{-1} for pure water).

Scattering spectra of the same fruits are represented in the right pane. There is no particulars spectral features but for a slight decrease of MUSP upon increasing the wavelength. In terms of absolute values, higher MUSP are observed in the Starking apples 13÷22 cm^{-1}), intermediate ones for the Golden apples (12÷15 cm^{-1}), while lower scattering values are observed for the Granny-Smith apples (8÷13 cm^{-1}). Some internal variations are observed also among different fruits of the same variety (e.g. Granny-Smith apples), or different positions on the same apple (e.g. Starking apples). There seems to be no particular correlation between the CHL content and the scattering spectra.

The influence of the skin on the result of a TRR measurement is shown in Figure 3. Absorption (left pane) and scattering (right pane) spectra were obtained on a Golden apple (top row), a Granny-Smith apple (middle row), and a Starking apple (bottom row). For each fruit, two measurements were performed, before, and after skin removal. In both cases the fibers were positioned in the very same point to probe the same region. The spectra obtained for the whole apples (filled diamonds) are quite overlapped to the spectra for the peeled apples (empty diamonds) both for absorption

and scattering. Thus, it is possible to infer that the TRR measurement probes the optical properties of the pulp of the fruit regardless of the skin color.

In a further experiment, we checked the penetration depth of a TRR measurement. It is well known that the volume probed by a TRR measurement is a "Banana shaped" region connecting the injection and collection points[17]. It is not easy to define the measurement volume, since the photons paths are more densely packed around the banana region but can be distributed in the whole medium. Thus, we tried to determine the maximum depth in the pulp that can give some detectable contribution to the TRR curve. A series of measurements were performed on a Granny-Smith apple cutting slices of pulp on the opposite side of the fibers. Spectra were taken on the whole apple, and then removing slices so to yield a total thickness of 4.1, 2.7, 2.1, and 1.5 cm. The fitted absorption and scattering spectra are shown in Fig. 4. For what concerns the absorption measurement, the μ_a-value is unchanged down to a thickness of 2.7 cm. For 2.1 cm μ_a starts deviating from the measurement on the whole apple with a discrepancy of 25% at 675 nm, while for a thickness of 1.5 cm the discrepancy increases up to 50%. The highest variations are observed on the tails of the spectrum, where absorption is lower. The results on the scattering coefficient show a similar behavior, with almost no changes down to a thickness of 2.7 cm, and discrepancies of 15% and 25% for a 2.1 and 1.5 cm thickness, respectively. Overall, these results show that the TRR measurement is probing a depth of at least 2 cm in the pulp. Of course this is a rough estimate, yet it proves that the TRR measurement is not confined to the surface of the fruit. Moreover, the penetration depth can be somehow dependent on the optical properties of the fruits, and we expect deeper penetration for lower absorption and/or lower scattering.

To prove the sensitivity of the technique, we followed the change in chlorophyll absorption for apples after storage at room temperature. Two golden apples at different ripeness grades were measured at different times (time 0, and after 4 day storage at room temperature). The results are reported in Fig. 5. There is a clear decrease in the absorption coefficient, related to a decrease of the CHL content, as evident from both apples. Under the experimental conditions proposed in this study, the TRR technique is capable of tracking a relative change of μ_a up to a precision of 2%[16]. Thus, we expect a high sensitivity in monitoring internal processes of the fruit that involve changes in the absorption coefficient.

Discussion

In this work we have shown the applicability of TRR for the non-destructive and non-invasive measurement of the optical properties in apples. A key point of the technique is the independence of the measurement from the color of the skin. In-fact, a thin absorbing layer behaves like an attenuating filter, without affecting the TRR curve. At a given point on the fruit surface, the intensity of re-emitted light is decreased due to the skin absorption, but the shape of the time-dispersion curve is minimally affected since photons do not travel much into the skin.

Photons involved in a TRR measurement probe a region of the pulp of the fruit with a typical banana shape[15]. Thus, the measured coefficients roughly correspond to the average of the optical properties of this probed region. In particular, for the tested Granny-Smith apple, that is for $\mu'_s \approx 18$ cm^{-1}, and $\mu_a \approx 0.07$ cm^{-1} at 675 nm, the depth of the probed region is about 2 cm, that is well below the skin. For lower values of μ_a (e.g.

on the tails of the CHL absorption peak) the probed region is even wider, as appears from Fig. 5, where the highest discrepancy is observed on the tails of the spectrum.

We have used the TRR technique to measure the absorption and scattering properties of different apple varieties in the spectral region around the CHL absorption peak. The technique can be applied also to other turbid fruits or vegetables (for instance, we have measured pears, peaches, nectarines, melons, kiwis, and tomatoes), while it can not be used whenever scattering is too low (e.g. orange) or the fruit too small (e.g. strawberries).

The use of the optical properties of the pulp of fruits and vegetables for the assessment of the fruit internal quality has still to be investigated. More studies are needed trying to correlate the measured optical properties to other chemicals or physical parameters of the fruit such as soluble solids (sugar), acidity or firmness. Since TRR permits the measurement of the absorption spectrum of the pulp independent of the scattering properties, a challenging task could be the detection of absorbing substances like CHL, and anthocyanins in the visible or sugar and water in the near infrared region. Also, this technique can be tried to follow-up the ripening process, or to monitor fruit changes during long-term storage. On the other hand scattering inside a fruit is mainly due to refraction index mismatches among liquids and membranes. Thus, the mean scattering coefficient can provide information on the internal structure, as suggested also by a recent study on kiwis[18]. Changes in the scattering coefficient can be strictly related to the ripening process on one side, and on the aging mechanism on the other one.

A possible criticism against the usefulness of TRR for applications in agriculture is the cost and complexity of the instrumentation, especially whenever more than one wavelength is needed. Nonetheless, the fast progress in optoelectronics and mainly in telecommunications has led to a rapid growth of instrumentation for time-resolved measurements, so that the development of a compact and low-cost time-resolved instrument is not out of sight. Indeed, we have built a first prototype, working with semiconductor lasers, a compact photomultiplier and all-fiber optics that can be used as a stand alone and portable instrument.

Conclusions

In conclusion, we have demonstrated for the first time the feasibility of a TRR measurement on apples for the non-invasive detection of the absorption and reduced scattering coefficients. Using an interfiber distance of 1.5 cm, the measurement is sensible to the optical properties of the pulp down to a depth of approximately 2 cm, and is not influenced by the skin. In the wavelength range from 610 nm up to 700 nm the technique is able to reveal the absorption spectrum of CHL. Work is in progress to ascertain the use of the pulp optical properties for the assessment of quality related parameters or of the ripeness grade.

Acknowledgments

The work was partially supported by the EC grant FAIR CT96-1060.

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Figure Captions

Fig.1 Typical fit of a time-resolved reflectance curve. Experimental data (dots) are fitted with the convolution (continuous line) of the instrumental transfer function (dashed line) with a theoretical model (not shown).

Fig.2 Absorption (left pane) and scattering (right pane) spectra of Golden apples (top row), Granny-Smith apples (middle row), and Starking apples (bottom row). For each variety, a ripe fruit (triangles) and a greener one (diamonds) were selected. Two spectra were taken on each fruit from the side more exposed to sunlight (filled symbols) and from the opposite one (empty symbols).

Fig.3 Absorption (left pane) and scattering (right pane) spectra of a Golden apple (top row), a Granny-Smith apple (middle row), and a Starking apple (bottom row). Spectra were obtained before (filled diamonds), and after (empty diamonds) skin removal. No major changes are observed after peeling the fruits, demonstrating that the TRR measurement is unaffected by the skin.

Fig.4 Absorption and scattering spectra of a Granny-Smith apple. Different curves correspond to measurements on the whole apple, and on slices of the same apple obtained by cutting the fruit on the opposite side of the optical fibers.

Fig.5 Changes in the absorption spectrum due to aging. A green apple (triangles) and a ripe apple (diamonds) were measured at time 0 (filled symbols) and after 4 days of storage at room temperature (empty symbols).

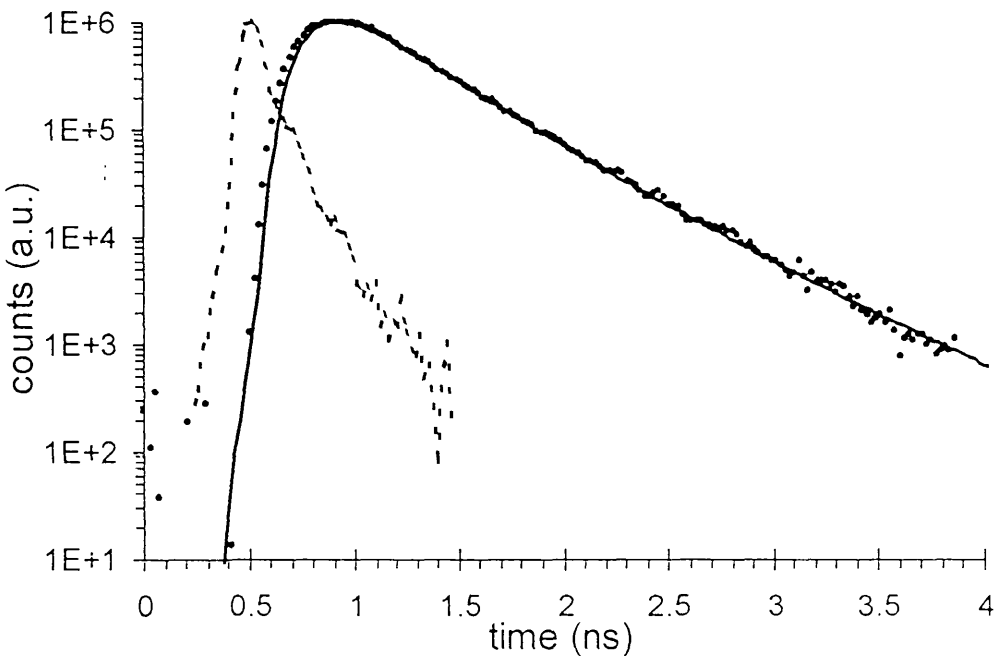
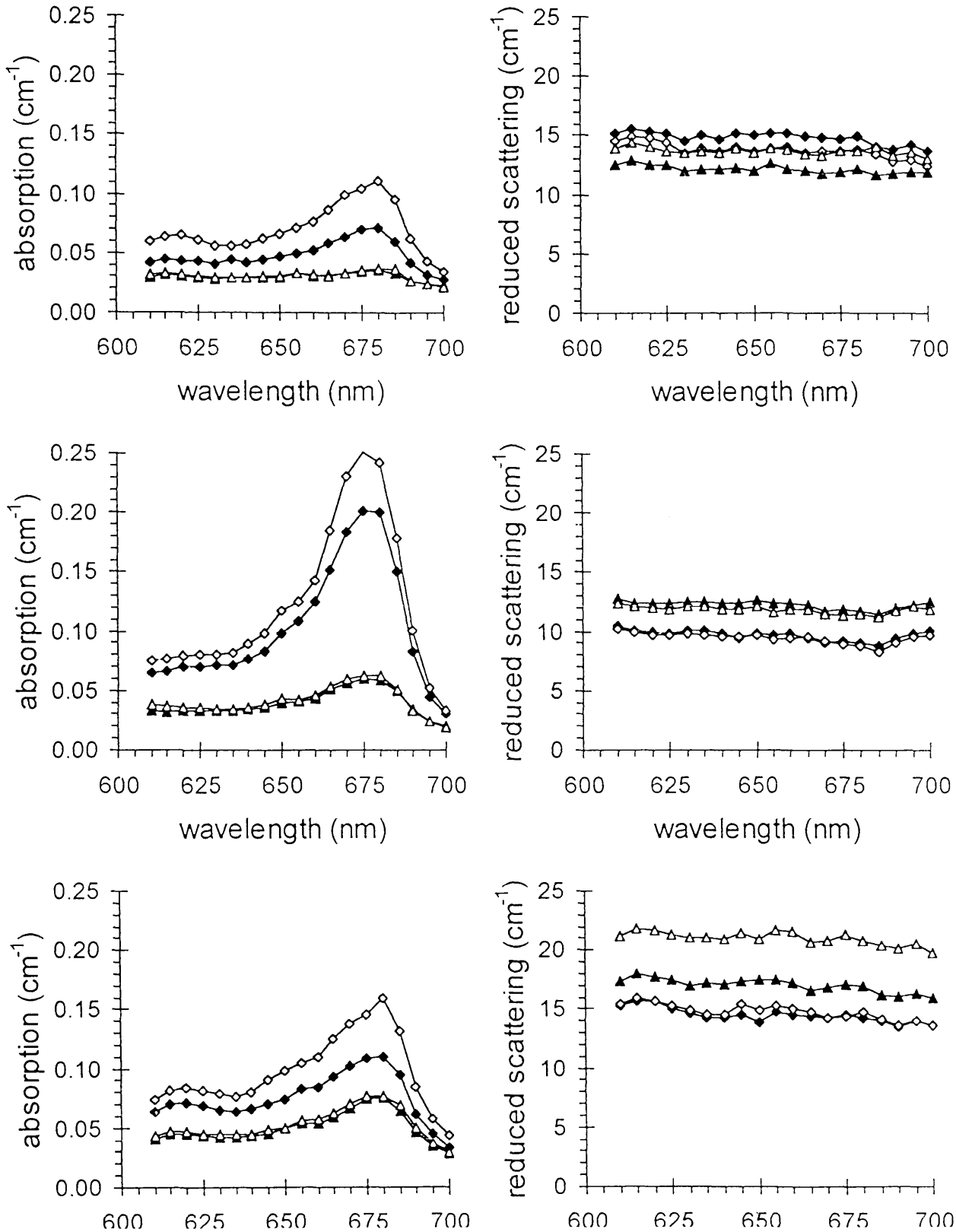


Figure 1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reduced scattering (cm-1)
wavelength (nm)
0.25
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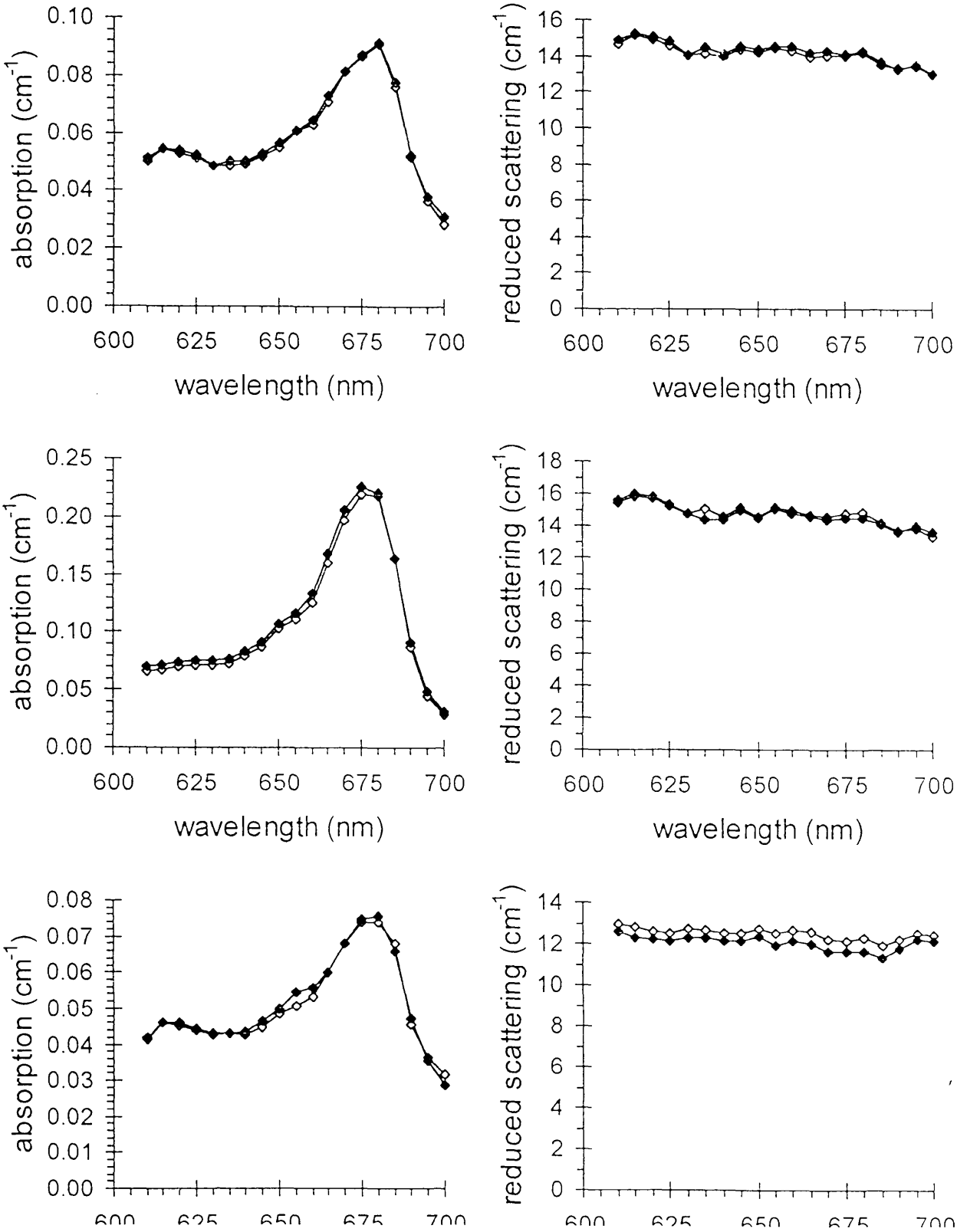
0.15
0.10
0.05
0.00
25
20
15
10
5
0
600
625
650
675
700

Figure 2


absorption (cm-1)
0.10
0.08
0.06
0.04
0.02
0.00
600 625 650 675 700
wavelength (nm)
reduced scattering (cm-1)
16
14
12
10
8
6
4
2
0
600 625 650 675 700
wavelength (nm)
absorption (cm-1)
0.25
0.20
0.15
0.10
0.05
0.00
600 625 650 675 700
wavelength (nm)
reduced scattering (cm-1)
18
16
14
12
10
8
6
4
2
0
600 625 650 675 700
wavelength (nm)
absorption (cm-1)
0.08
0.07
0.06
0.05
0.04
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reduced scattering (cm-1)
14
12
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Figure 3

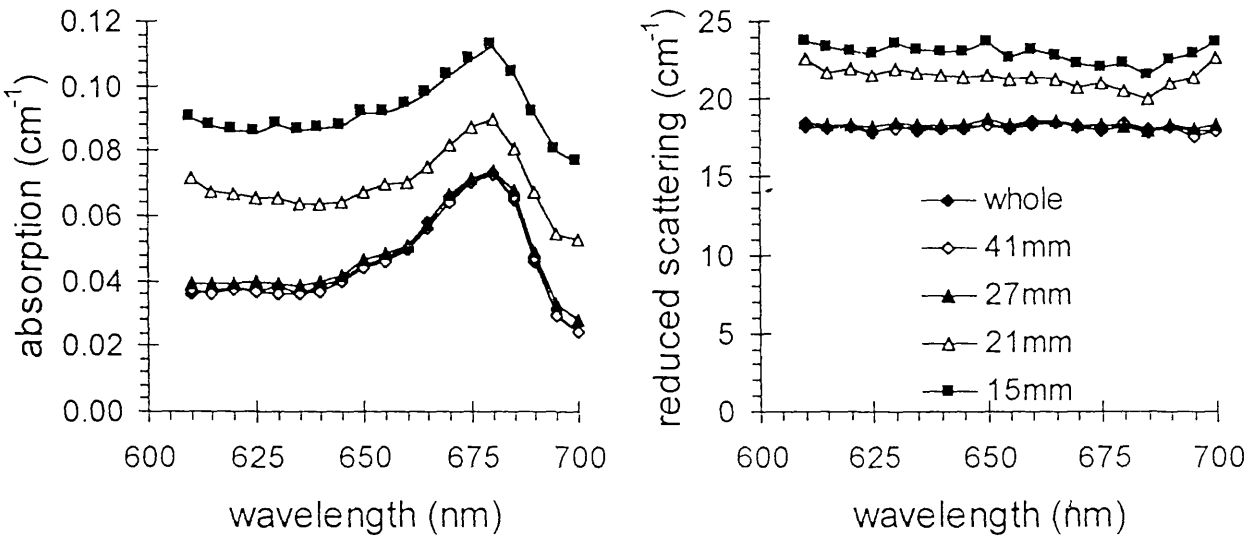
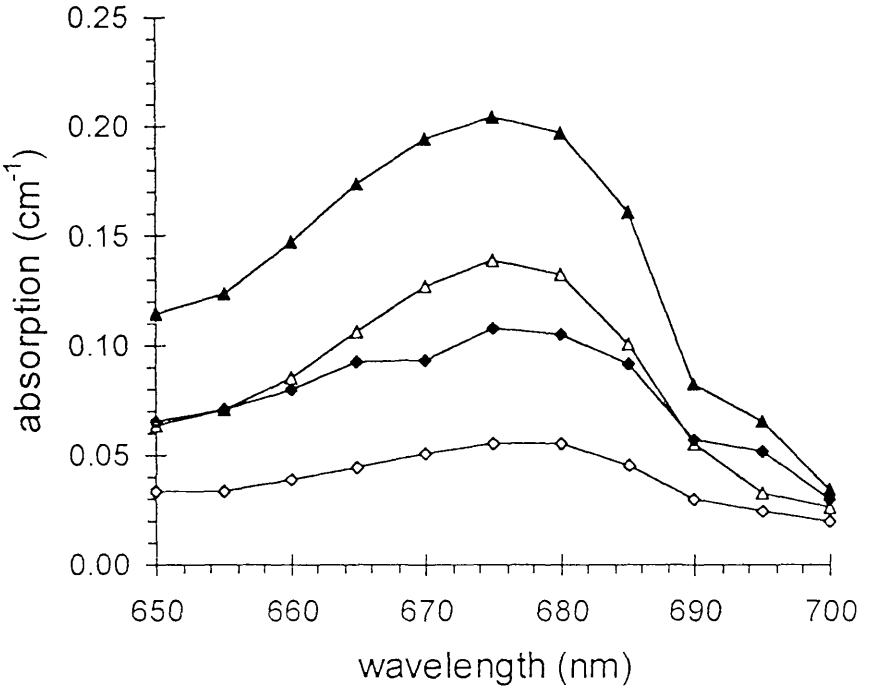

absorption (cm-1)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
600
625
650
675
700
wavelength (nm)
reduced scattering (cm-1)
25
20
15
10
5
0
whole
41mm
27mm
21mm
15mm
600
625
650
675
700
wavelength (nm)

Figure 4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0.20
0.15
0.10
0.05
0.00
absorption (cm-1)
650
660
670
680
690
700
wavelength (nm)

Figure 5