propylenes. We suggest that the latter may reflect an isotope dependence on the rotational degrees of freedom available to the pendant methyl groups.

In conclusion, deuterium substitution influences lattice spacings in hexatriacontane, and presumably other nonpolar polymers, in a predictable way. We believe that the underlying changes in molecular volume and polarizability, which have been previously shown to lead to nonideal mixing in binary liquid isotopic polymers mixtures,[1-4] are also primarily responsible for the melting temperature isotope effect in these systems.

Registry No. $C_{36}H_{74}$, 630-06-8; $C_{36}D_{74}$, 16416-34-5; $[CH_2]_n$, 9002-88-4; $[CD_2]_n$, 25549-98-8; $[C_8H_8]_n$, 25086-18-4; $[C_8D_8]_n$, 108765-83-9; $[C_3H_6]_n$, 25085-53-4; $[C_3D_6]_n$, 108073-51-4.

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The Effect of Molecular Size on the Absorption Spectra of Macromolecules

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ABSTRACT: The applications of the theory of scattering of electromagnetic radiation to the estimation of the absorption coefficients of macromolecules yield values for the absorption coefficients that are considerably different from those obtained from the direct application of Beer-Lambert law. The differences in absorption coefficients can be explained in terms of the size and the optical properties of the macromolecules. This paper reports on the effects of the molecular size on the absorption spectra of macromolecules using as a basis the theory of electromagnetic radiation. As a result from this investigation, the conditions for the strict correspondence between Mie theory and Beer-Lambert law have been derived. A sensitivity analysis is conducted to define the limits of application of Beer-Lambert law and the conditions required to extract size information from transmission measurements in the region of intrinsic chromophore absorption. As case studies, the UV/vis spectra of well-characterized polystyrene standards have been investigated.

Introduction

The primary purpose of this paper is to examine in detail the effect of the molecular size on the absorption spectra of macromolecules in solution. This is achieved theoretically, through the use of the theory of electromagnetic radiation, and experimentally, through careful analysis of commercially available narrow molecular weight distribution polystyrene standards. The motivation for this study stems from the discrepancy found in literature reported values of the absorption coefficients of macromolecules and on the implications of such differences for the estimation of polymer compositions and polymer concentrations. The results from the investigation are particularly relevant to the characterization of macromolecules using spectroscopy techniques.

Absorption spectroscopy techniques are routinely used to investigate reaction kinetics and to determine the concentration and composition of macromolecules in chromatographic effluents. In these cases the spectra are recorded at frequencies where at least some of the chromophores present absorb strongly. According to Bouger and later to Lambert,[1] the decrease in intensity of incident light of any given wavelength on passage through an absorbing medium can be described as a first-order process proportional to the thickness (l) of the absorbing material[1,2]

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$$\ln I_t/I_0 = -Kl \tag{1}$$

where I represents the intensities of the incident light (0) and the transmitted light (t). The proportionality constant K, known as either the absorption or the extinction coefficient, is characteristic of the absorbing material at the frequency of the incident radiation. In 1852 Beer established that, for homogeneous solutions containing chromophores, the decrease in intensity is directly proportional to the concentration of chromophores (C) and to the length of the absorbing path.[1,2]

$$\ln I_t/I_0 = -ECl \tag{2}$$

The new proportionality constant E is the molar absorption coefficient. Equation 2, known as Beer–Lambert law, is used extensively for the quantification of chromophores in solution. The validity of Beer–Lambert law hinges upon several assumptions: (i) the incident radiation is monochromatic; (ii) the decrease in intensity is due only to absorption; (iii) absorption is limited to a volume of uniform cross section; and (iv) each absorbing center is independent of all others regardless of their kind and concentration. In many cases, the deviations from Beer–Lambert law can be attributed to failures in one or more of the above assumptions. When Beer–Lambert law is applied to macromolecules and colloids, assumptions ii and iv are of primary concern because of the finite size of the macromolecules and the close proximity of functional groups within the molecule.

The size of the molecules will produce deviations which are due, primarily, to the combined effects of absorption and scattering whereas the close proximity of neighboring functional groups will affect the electron mobility and hinder intramolecular motions. The effect of neighboring groups on the absorption spectra can be treated by considering the affected groups as independent chromophores with absorption patterns that depend on the nature of the neighboring groups.[3,4] The effects of the molecular size on the attenuation are more difficult to analyze because of the combined effects of absorption and scattering. There is theoretical and experimental evidence to suggest that the absorption coefficient of macromolecules is sensitive to the molecular weight[5,6] and to the state of aggregation of the solute.[7,8] In this paper it is proposed that the observed sensitivity of the UV/vis spectra to the molecular weight of macromolecules in solution can be explained in terms of Mie theory.

Experimental Methods

Narrow polystyrene standards (Scientific Polymer Products, Inc.) were dissolved in spectral grade tetrahydrofuran (Burdick and Jackson Laboratories) at several carefully predetermined concentrations. The UV/vis spectra were recorded in a Perkin-Elmer 3840 photodiode array UV/vis spectrophotometer equipped with a thermoelectric cell holder and a temperature controller with temperature programming capabilities. A 1-cm path length cell was used for all measurements. The turbidity spectra of the polystyrenes were obtained from measurements at several concentrations. Up to seven concentrations plus replicates were used in order to obtain good estimates of the optical constants as well as good estimates of the measurements errors. Special care was taken to ensure that the measurements were always within the linear range of the instrument. The raw spectroscopy data was stored in a Perkin-Elmer 7500 computer and analyzed by using the interpretation software developed in-house.

Adaptation of Mie Theory to the Spectra of Macromolecules in Solution

Mie theory represents the ideal scattering behavior expected of mixtures containing particles, and although macromolecules are not, in general, spherical or isotropic, Mie theory constitutes a reasonable starting point for the analysis, particularly, because it gives rise to general scattering models with the smallest number of parameters. In order to apply Mie theory to the analysis of homogeneous solutions of macromolecules, it is necessary to deviate from the continuum representation of homogeneous solutions and consider that, relative to the size of the solvent molecules, macromolecules in solution form an optically heterogeneous system where the discrete entities are the macromolecules and the continuum is the solvent. In this context the terms macromolecules and particles will be used interchangeably. Under these conditions, if absorption and scattering are present, the logarithm of the ratio of transmitted to incident radiation is known as turbidity (τ). For dilute solutions, it has been shown that the turbidity is given by[9-11]

$$\tau = \ln I_0/I_t = N\gamma l \tag{3}$$

where N is the number of particles/milliliter and γ represents the extinction due to absorption and scattering. Notice that eq 1–3 have the same functional form. Therefore, the deviations from the expected behavior eq 1–3 are related to the interpretation of the corresponding proportionality constants (i.e., K, E, and γ).

In typical transmission spectroscopy experiments there are three major sources of intensity losses: absorption by the suspending medium, absorption by the macromolecules, and scattering by particles or macromolecules. The contribution of the particle absorption and scattering to the overall spectra can be quantified, in principle, from Mie theory. Mie theory relates the measured turbidity to the number, size, and optical constants of suspended isotropic spherical particles

$$\tau(\lambda) = Nl \int_0^\infty \frac{\pi}{4} D^2 Q(\alpha,m) f(D)\, \mathrm{d}D \tag{4}$$

or

$$\tau(\lambda) = \frac{3Cl}{2\rho} \frac{\int_0^\infty D^3 Q(\alpha,m) f(D)\, \mathrm{d}D}{\int_0^\infty D^3 f(D)\, \mathrm{d}D} \tag{5}$$

$$\alpha = \pi D/\lambda$$

$$m = (n_1 + ik_1)/n_0 = n + ik$$

where $\tau(\lambda)$ is the turbidity at the wavelength λ, C the concentration in g/mL, D the particle diameter, $f(D)$ the frequency distribution of particle sizes, ρ the density of the particles or the molar density of the macromolecules, and m the complex refractive index ratio. The optical parameters n_1 and k_1 represent the real and the imaginary parts of the complex refractive index for the macromolecules whereas n_0 represents the real part of the complex refractive index for the solvent. $Q(\alpha,m)$ represents Mie's overall extinction efficiency, and it is a well-known complex function of α and m (see for example van de Hulst,[9] Kerker,[10] and Bohren and Huffman.[11] Notice that the extinction coefficient γ in eq 3 is now a function of the size and the optical properties of the macromolecules and the suspending medium.

The direct analysis of eq 4 or 5 is rather difficult, particularly if the form of the particle size distribution is unknown. In order to investigate the effect of the molecular size (i.e., the particle diameter), a monodisperse

Table I
Coefficients for the Fifth-Order Power Series Approximation to the Extinction Efficiency[12]

$$\Gamma_1' = \frac{24nk}{(n^2 + k^2)^2 + 4(n^2 - k_2) + 4}$$

$$\Gamma_3' = \frac{4nk}{15} + \frac{20nk}{3[4(n^2 + k_2)^2 + 12(n^2 - k_2) + 9]} + \frac{4.8nk[7(n^2 + k_2)^2 + 4(n^2 - k_2 - 5)]}{[(n^2 + k^2)^2 + 4(n^2 - k^2) + 4]^2}$$

$$\Gamma_4' = \frac{8}{3}\,\frac{[(n^2 + k_2)^2 + (n^2 - k^2 - 2)]^2 - 36n^2k^2}{[(n^2 + k^2)^2 + 4(n^2 - k^2) + 4]^2}$$

$$\Gamma_5' = \frac{6}{175}(P_1R_2 + P_2R_1) - \frac{20nk[4(n^2 + k^2)^2 + 8(n^2 - k^2) + 21]}{21[4(n^2 + k^2)^2 + 12(n^2 - k^2) + 9]^2} + \frac{2}{225}\left(\frac{14nk}{9(n^2 + k^2)^2 + 24(n^2 - k^2) + 16}\right) + \frac{2}{315}(2nk + 4nk(n^2 - k^2 - 3))$$

$$P_1 = \frac{(n^2 + k^2)^2 + n^2 - k^2 - 2}{Z}$$

$$P_2 = \frac{6nk}{Z}$$

$$R_1 = \frac{(n^2 - k^2)^4 + 22(n^2 - k^2)^3 - 160(n^2 - k^2)^2 - 200(n^2 - k^2)}{Z} + \frac{400 - 4n^2k^2[24(n^2 - k^2) + 4n^2k^2 + 39]}{Z}$$

$$R_2 = \frac{2nk[2(n^2 - k^2)^3 - 12(n^2 - k^2)^2 + 205(n^2 - k^2)}{Z} + \frac{8n^2k^2(n^2 - k^2 + 9) - 198]}{Z}$$

$$Z = (n^2 + k^2)^2 + 4(n^2 - k^2) + 4$$

The ith Γ Coefficient in Eq 6 and 10 is given by $\Gamma_i = \Gamma_i'(\pi/\lambda)^i$.

suspension of isotropic spherical particles will be assumed. By first considering the Rayleigh regime (i.e., where $D \ll \lambda$) and expanding $Q(\alpha,m)$ in power series of D, eq 4 can be approximated by

$$\tau(\lambda) = \frac{3Cl}{2\rho D^3}[\Gamma_1 D^3 + \Gamma_3 D^5 + \Gamma_4 D^6 + \Gamma_5 D^7 + \ldots] \quad (6)$$

where the values for the Γ coefficients are given in Table I. The coefficients for fifth-order approximation were first derived by Penndorf.[12] Higher order approximations have been obtained at our laboratories by using Macsyma.[13]

The effects due to scattering can be eliminated from eq 6 by assuming an isorefractive system (i.e., $m = 1 + ik(\lambda)$). An additional simplification can be introduced by noticing that, for most macromolecules, the value of k_1 at $\lambda_{\max}$ in the region of interest (i.e. 230–400 nm) is of the order of 10^{-4}. Under these conditions the coefficients given in Table I can be readily evaluated to yield

$$\tau(\lambda) = \frac{Cl\epsilon(\lambda)}{\rho}[1 - (\pi/3\lambda)^2\epsilon(\lambda)D^3 - \ldots] \quad (7)$$

where $\epsilon(\lambda) = 4\pi k/\lambda$. Equation 7 describes the spectrum of a suspension of isotropic spherical particles with diameter D in an isorefractive medium. Note that in the limit of $D \rightarrow 0$ the absorption coefficient in eq 7 is identical with the absorption coefficient in eq 2 (i.e., $E = \epsilon(\lambda)$). That is, according to eq 7, Beer–Lambert law (eq 2) is a special case of Mie theory. E and $\epsilon(\lambda)$ are strictly equivalent only for very small particles in an isorefractive medium.

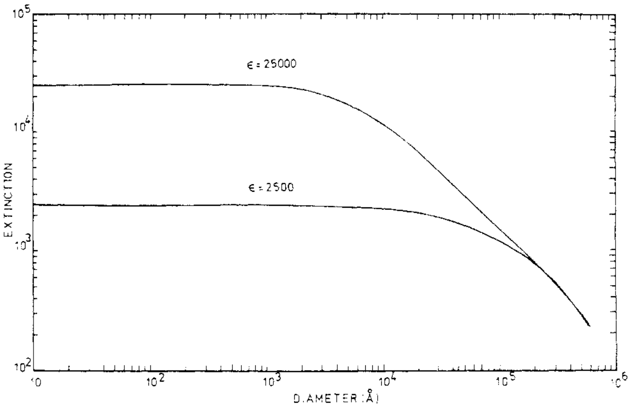

Figure 1. Calculated effect of the molecular size on the extinction coefficient for an isorefractive system. The absorption coefficients used to generate the data are indicated: $\lambda_0 = 254$ nm.

Equation 7 also indicates that, for the same mass (i.e., concentration), if the state of aggregation changes (i.e., by an increase in the particle diameter) there will be a concomitant change in the observed turbidity even in the absence of scattering. For example, if a true isorefractive solution obeying Beer–Lambert law aggregates, the observed turbidity will be a monotonically decreasing function of the diameter of the aggregates. This can be clearly seen by taking the ratio of the turbidity due to the suspension to that predicted in the limit of zero size

$$R(\lambda) = 1 - (\pi/3\lambda)^2\epsilon(\lambda)D^3 - \ldots \quad (8)$$

In scattering situations (i.e., $n = n_1/n_0 \neq 1$), where the particle diameters are small enough such that the terms containing D can be neglected, eq 7 can be readily reduced to

$$\tau(\lambda) = \frac{Cl}{\rho}\left[\frac{9n\epsilon(\lambda)}{(n^2 + 2)^2}\right] = Cl\epsilon'(\lambda) \quad (9)$$

Notice that eq 9 also has the same form as Beer–Lambert law where now the effective absorption coefficient $\epsilon'(\lambda)$ is related to the absorption coefficient $\epsilon(\lambda)$ through the real part of complex refractive index ratio. Clearly, the absorption coefficient generally estimated in the limit of $D \rightarrow 0$ is $\epsilon'(\lambda)$.

At this point it is important to recapitulate on the interpretation of the coefficients K, E, γ, $\epsilon(\lambda)$, and $\epsilon'(\lambda)$ appearing in eq 1, 2, 7, and 9. K and E are often interpreted as absorption coefficients, however, in the context of Mie theory they represent the total extinction due to turbidity resulting from the combined effects of absorption and scattering (i.e., γ). In the limit of zero size and for an isorefractive solution, the extinction coefficient E is identical with the absorption coefficient $\epsilon(\lambda)$ defined in terms of the imaginary part of the complex refractive index ratio k. In the limit of zero size, if the solvent is not isorefractive, the total extinction (E) becomes identical with the effective absorption coefficient $\epsilon'(\lambda)$ where the effective absorption coefficient is now a function of the real and the imaginary parts of the complex refractive index ratio. Notice that ϵ and ϵ' are meaningful only at very small molecular (or particle) sizes where the high order terms in eq 9 can be neglected. It is evident that, the relevant optical parameters are the real and imaginary parts of the

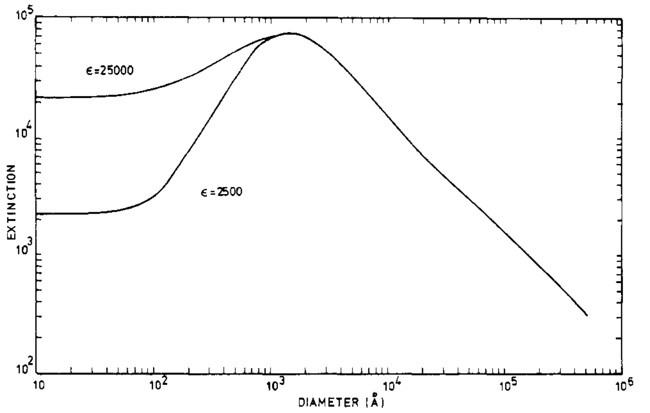

Figure 2. Calculated effect of the molecular size on the extinction coefficient of a system typical of macromolecules in solution. The absorption coefficients used to generate the data are indicated: λ_0 = 254 nm; n_0 = 1.45; n_1 = 1.74.

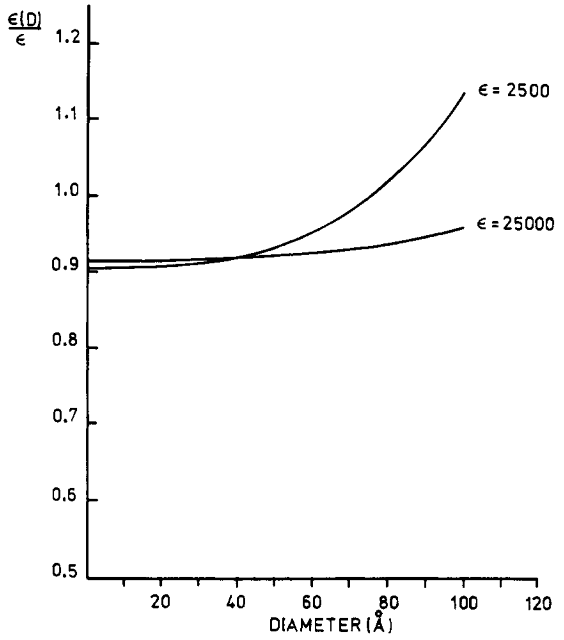

Figure 3. Extrapolation to zero size of the normalized values of the expected extinction coefficient of a system typical of macromolecules in solution. The absorption coefficients used to generate the data are indicated: λ_0 = 254 nm; n_0 = 1.45; n_1 = 1.74.

complex refractive index ratio (see the Γ coefficients given in Table I).

Notice that interpretation of ϵ' as if it were ϵ is a source of error since the values of k_1 obtained from $\epsilon'(\lambda)$ will not be able to reproduce the measured spectra. It is also important to notice that the scattering effects are not additive to the absorption coefficients ϵ and ϵ'. The additive properties are the absorption and scattering efficiencies which in turn are complex functions of the optical parameters k and n.

For polydisperse systems, replacement of the power series expansion of $Q(\alpha,m)$ into eq 4 results in the expansion of the turbidity in terms of the moments of the particle size distribution[14]

$$\tau(\lambda) = \frac{3Cl}{2\rho\bar{D}_3}[\Gamma_1\bar{D}_3 + \Gamma_3\bar{D}_5 + \Gamma_4\bar{D}_6 + \Gamma_5\bar{D}_7 + ...] \qquad (10)$$

where $\bar{D}_i$ represents the ith moment of the particle size distribution. Notice from eq 10 that the effect of the molecular size will be similar for monodisperse and polydisperse systems. For polydisperse systems, the larger diameters will have a more dramatic impact on the spectra since they have a large influence on the higher moments of the distribution. The variance of the distribution will determine the magnitude of such effects.

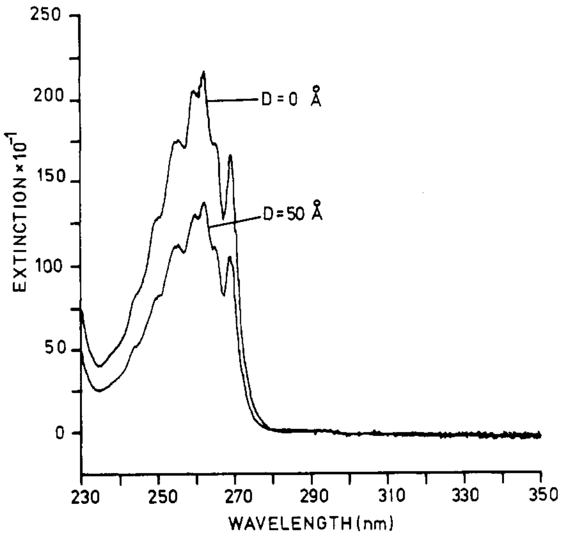

Figure 4. Calculated effect of 50-Å particles on the extinction spectra $n_0\epsilon(\lambda)$ of polystyrene in tetrahydrofuran (D = 0 Å). The spectra at 50 Å is the expected experimental measurement if the small size extrapolation is used (i.e., $\epsilon'(\lambda)$). Notice that the spectrum for D = 0 has been plotted as $n_0\epsilon(\lambda)$ to enhance the differences.

The upper limit on the particle diameter for the application of eq 6 is approximately 500 Å at 200 nm (i.e., $\alpha \leq 0.8$) with the real part of the complex refractive index ratio restricted to values less than 2.[12] The limit of applicability for eq 10 is dictated by the magnitude of the moments $\bar{D}_1$, $\bar{D}_2$, etc. In order to extend the range of application of eq 6 and 10 higher order terms in the power series expansion of the extinction efficiency may be added, the extinction efficiency may be expanded by using Pade approximations[16] or, alternatively, the extinction efficiency may be calculated numerically by using available computer codes.[17,18]

Numerical Analysis

The effect of the particle size on the expected turbidity for a nonscattering isorefractive medium is shown in Figure 1. The curves were calculated from eq 5 and 7 by using values typical of polymers and proteins (i.e., $\epsilon(\lambda)$ = 2500 and 25 000 cm^2/g at 254 nm). The curves suggest that, initially, there is a small decrease in intensity (not noticeable in a logarithmic scale) followed by rapid drop in turbidity due to the increase in molecular size. As the absorption coefficient increases, the rapid drop in turbidity is displaced to smaller sizes. It is apparent that the extrapolation to zero size will provide a reasonable estimation of the absorption coefficient $\epsilon(\lambda)$. The nonlinear nature of Mie theory can be readily appreciated in Figure 2 where the effect of the refractive index has been included in the calculations (i.e., n_1 = 1.74 and n_0 = 1.45). For this case there will be a maximum turbidity as a function of the molecular size. The position of the maximum depends, primarily, on the real part of the complex refractive index ratio. To the right of the maximum, the information content on the absorption coefficient decreases, whereas to the left of the maximum, the information content on the particle size decreases. As the particle size decreases, a plateau is reached where there is negligible effect of the molecular size. It would appear that measurements of the absorption coefficient in this region would also yield good approximations to $\epsilon(\lambda)$. Close inspection of the results

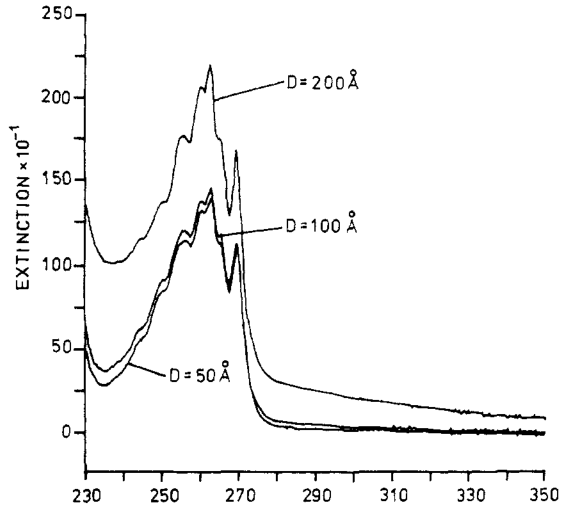

Figure 5. Calculated effect of the molecular size on the extinction spectra of polystyrene in tetrahydrofuran (i.e., $\epsilon(\lambda)$). The molecular diameters used in the calculations are indicated.

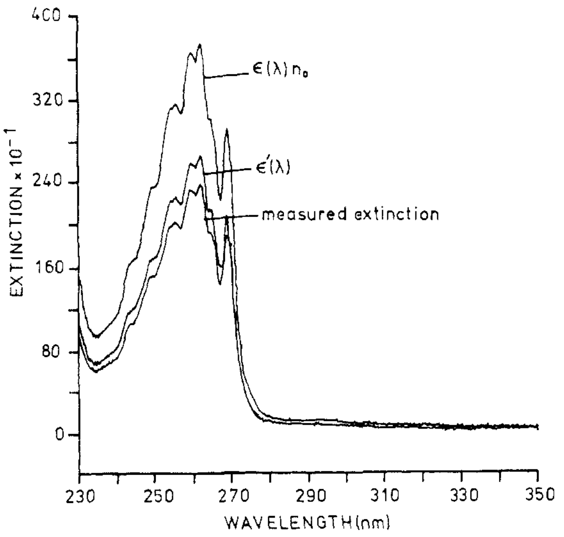

Figure 6. Calculated effect of the molecular size on the extinction spectra of polystyrene in tetrahydrofuran (i.e., $\epsilon(\lambda)$). The molecular diameters used in the calculations are indicated.

shows that the estimated absorption coefficients would be 2260 and 22 610 whereas the values used to generate the data were 2500 and 25 000, respectively. The differences in the absorption coefficients can be better appreciated in Figure 3 where the absorption coefficients normalized to $\epsilon(\lambda)$ have been plotted in a linear scale. Notice that direct extrapolation to zero size will not yield the desired value for the absorption coefficient $\epsilon(\lambda)$, rather $\epsilon'(\lambda)$ will be obtained. The difference between $\epsilon(\lambda)$ and $\epsilon'(\lambda)$ is proportional to the real part of the complex refractive index ratio (eq 9).

The effect of the molecular size over the complete absorption spectrum is shown in Figures 4–6. Note that, initially, the expected extinction is smaller than the absorption coefficient $\epsilon(\lambda)$ (Figure 4). As the molecular size increases, the relative importance of scattering over absorption increases with a concomitant increase in the expected turbidity (Figure 5). Notice that as the size increases, the spectra become distorted and the fine structure in the spectra vanishes (Figure 6). Figures 4–6 were calculated by using the absorption coefficients $\epsilon(\lambda)$ from the measured spectrum for a narrow polystyrene standard and the refractive index dispersion equations for polystyrene (Bateman et al.[18])

$$n_1 = 1.5683 + 10.087 \times 10^{-11}/\lambda_0^2$$

and tetrathydrofuran as solvent (Huglin[18])

$$n_0 = 1.3947 + 3.5562 \times 10^{-11}/\lambda_0^2$$

where λ_0 is given in centimeters. Clearly, on the basis of the optical constants used, molecules having equivalent diameters smaller than 100 Å are expected to show negligible effects of the molecular size on the absorption coefficients. The only expected discrepancy in reported values of the absorption coefficients would arise from the misinterpretation of ϵ' as ϵ. Molecules having equivalent diameters larger than 100 Å are expected to show significant differences in turbidity.

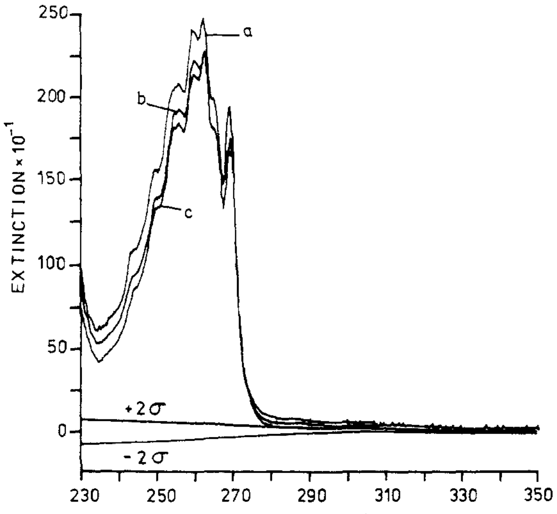

Figure 7. Measured spectral differences between low and high molecular weight polystyrenes in tetrahydrofuran: (a) M_w 100 000; (b) M_w 50 000; (c) M_w 13 000.

Experimental Results

The experimental determination of the extinction for narrow polystyrene standards confirms, qualitatively, the calculated results. Figure 7 shows the differences in extinction for several polystyrene standards as functions of the molecular weight. Evidently, the molecular size will bias the estimation of polymer concentrations whenever polymer concentrations are determined, but just as importantly, the estimation of polymer compositions will be biased to the extent that the samples compared are different in molecular weight. Note that the effect of the molecular size on the extinction appears at molecular weights smaller than those anticipated from the calculations. For example for a molecular weight of 1 million, Kerker estimated an equivalent particle diameter of 56 Å.[10] This discrepancy may be attributed to the lack of reliable values for the refractive index in the region of intrinsic chromophore absorption. Figure 8 shows that the experimentally observed differences in the absorption spectra, attributed to differences in molecular size, are significant at the 95% confidence level. If the molecular weight values reported by the manufacturer are considered valid, it is possible to calculate the absorption coefficients $\epsilon(\lambda)$ and $\epsilon'(\lambda)$ on the basis of the refractive indices reported in the literature.[18,19] Figure 9 shows the values for $\epsilon(\lambda)$ and $\epsilon'(\lambda)$ estimated from the measured extinction for the 100 000 molecular weight standard. As expected, the values for $\epsilon(\lambda)$ are larger than the measured extinction. The diameter

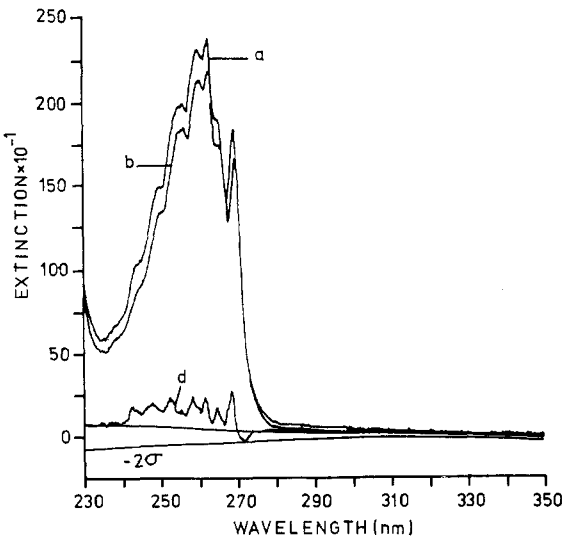

Figure 8. Measured spectral differences between two narrow polystyrene standards: (a) M_w 100 000; (b) M_w 13 000; (d) is the spectral difference between (a) and (b). The 95% confidence interval (i.e., 2σ level) on the measured spectra is indicated as reference.

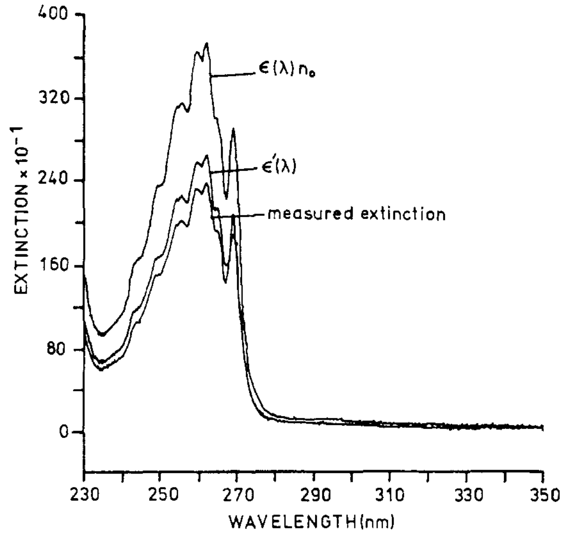

Figure 9. Estimated values for $n_0\epsilon(\lambda)$ and $\epsilon'(\lambda)$ for a 100 000 narrow polystyrene standard (D = 44 Å). The lower curve represents the measured spectra (see also Figure 3).

obtained, 44 Å, is in reasonable agreement with the values predicted on the basis of the molecular weights. The experimental observations are not surprising if it is remembered that Mie theory constitutes the basis for the determination of the molecular weights in the region where there is no absorption. As the wavelength is decreased and the region of intrinsic chromophore absorption is approached, stronger effects of the molecular size on the extinction spectra can be anticipated. The surprising aspect of the experimental results is that the molecular size has an effect on the extinction spectra of relatively low molecular weight polymers (i.e., Figure 7) suggesting that it may be possible to obtain better estimates of the molecular weights if the light scattering experiments are conducted in the region of intrinsic chromophore absorption. The added sensitivity will be at the expense of having to solve the combined scattering and absorption problem. However, in order to apply Mie theory, reliable estimates of the optical parameters k_1 and n in the region of intrinsic chromophore absorption are required. These optical constants, in particular the refractive indices, are currently being evaluated, and the results will be published separately.

Summary and Conclusions

The differences in the absorption coefficients of macromolecules reported in the literature have been explained on the basis of the theory of electromagnetic radiation. These differences may be due to size effects through combined absorption and scattering effects and/or to differences in the interpretation of the extinction coefficient in the limit of negligible size effects. The conditions for the strict correspondence between Mie theory and Beer–Lambert law have been presented together with a sensitivity analysis conducted to define both the limits of application of Beer–Lambert law and the conditions required to extract size information from transmission measurements in the region of intrinsic chromophore absorption. It was found that, on the basis of reported optical constants for polystyrene, Beer–Lambert law may be applicable to molecules having molecular sizes smaller than 100 Å. This value also suggests a range for the evaluation of the molecular size from transmission measurements. Careful experimental measurements for narrow polystyrene standards in the region of intrinsic chromophore absorption indicate that molecular sizes considerably smaller than 100 Å could be determined. However, for this purpose, better values of the optical constants in the range of intrinsic chromophore absorption are required.

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