EFFECT OF CHANGE IN CONCENTRATION UPON LENS TURBIDITY AS PREDICTED BY THE RANDOM FLUCTUATION THEORY

FREDERICK A. BETTELHEIM AND ERNEST L. SIEW
Chemistry Department, Adelphi University, Garden City, New York 11530

ABSTRACT Theoretical calculations were performed to predict how the light scattering intensity would change with changes in concentration in the gel state. The theory of light scattering was applied to a random distribution of hard spheres. The spherical particles with constant diameter were embedded in a medium having a different refractive index. The light-scattering intensities obtained as a function of concentration showed that in dilute solutions the scattered light intensity increases with concentration. However, in concentrated solution >0.1 or 0.2 volume fraction, the light-scattering intensity decreases with increase in concentration.

INTRODUCTION

The transparency of normal lens indicates the absence of large fluctuation in the refractive index within the lens fibers due to the even distribution of lens protein (Trokel, 1962; Philipson, 1973). Benedek (1971), using general light-scattering theory, predicted that the opacity of cataractous lens may be caused by high molecular weight protein aggregates. Using a dilute solution theory approximation so that the high molecular weight proteins could be considered as individual scatterers, he calculated that a molecular weight of 5×10^7 or higher will cause turbidity. Such high molecular weight protein aggregates have been found both in normal and cataractous lenses (Spector, 1972; Hoenders and Van Kamp, 1972; Jedziniak et al., 1975 and 1978).

Although the qualitative prediction of the dilute solution approximation pointed in the right direction, there is a certain difficulty in applying such an approximation to a gel like the lens. On the basis of the angular dependence of light-scattering patterns (Bettelheim and Paunovic, 1979), we proposed that the full Debye-Bueche (1949) theory of light scattering from inhomogeneous solids would be needed to understand cataractogenesis. A modification of this theory uses random fluctuations both in density and orientation (Stein et al., 1959); we found it useful for obtaining structural parameters that cause light scattering both in normal human lenses (Bettelheim and Paunovic, 1979; Siew et al., 1981 *a*) and in lenses with nuclear cataracts (Chylack et al., 1981; Siew et al., 1981 *b*; Bettelheim et al., 1981).

A comparison of the dilute solution approximation (Benedek, 1971) and the condensed phase theory (Bettelheim and Paunovic, 1979) yielded different predictions for the increase in turbidity as a function of an increase in the size of the scattering units (Bettelheim, 1979). Even greater differences are found when a change in the concentration of the scattering units is considered. The dilute solution approximation would predict a proportional increase in turbidity with increasing concentration of the scattering units. A preliminary study in which the position of the particles was restricted to a cubic lattice has shown that the condensed-phase theory predicts a decrease in the light-scattering intensity with an increase in concentration (Bettelheim and Siew, 1980). A model that Benedek (1971, part II) has used to understand the transparency and opacification of the cornea is also based on density fluctuations. Similar to our predictions for the lens, this model predicts that increased opacification will occur upon corneal swelling.

To account for cataractogenesis due to osmotic pressure–generated dilution, we calculated the light scattering intensity changes that occur when a condensed system with random particle distribution undergoes a concentration change. This paper describes the results obtained.

THEORY

The Debye-Bueche (1949) theory accounts for the scattering of nonpolarized light by inhomogenous solids or gels due to fluctuations in the density. The density fluctuation is expressed as fluctuation in the refractive index over domains that are comparable with the size of the wavelength.

$$I = k\,\eta^2 \int \gamma(r)\,(\sin hr/hr)\,r^2\,\mathrm{d}r \qquad (1)$$

where $h = (4\pi/\lambda)\,\sin\theta/2$; θ and λ being the scattering angle and the wavelength in the medium, respectively. $\gamma(r)$ is the correlation function with the boundary conditions $r = 0; \gamma(0) = 1$ and $r = \infty; \gamma(\infty) = 0$, and has the form $(r) = (\langle \eta_1\eta_2 \rangle / \eta^2)$. We assume that the physical basis of light scattering in lens is the random distribution of high-density protein aggregates in the low-density cytoplasmic fluid. Correlation exists for all pairs of volume elements separated by an r distance that have local refractive index deviations from the average η_1 and η_2. $\langle \eta_1\eta_2 \rangle$ is the average of $\eta_1\eta_2$. In our previous work (Bettelheim and Paunovic, 1979; Siew et al., 1981 *b*; Siew et al., 1981 *a*) we used the analytical expression of a two-phase model

$$\gamma(r) = \phi \exp\left[-(r/a)^2\right] + (1 - \phi) \exp\left[-(r/d)^2\right] \quad (2)$$

to which we fit the experimentally obtained correlation functions. These were obtained by a Fourier inversion of data of scattered light intensity both in normal lens sections (Bettelheim and Paunovic, 1979) as well as in cataractous lens sections (Siew et al., 1981 *b*). In Eq. 2 ϕ is the volume fraction of particles with high refractive index embedded in a matrix with a lower refractive index. a is the average size of the particles (correlation length), and d is the average separation of the particles. The amplitude factor η^2 in Eq. 1 gives the mean-squared deviation from the average refractive index $\bar{n}$.

The purpose of the present investigation is to calculate the change in the light-scattering properties of gels such as the lens when changes in concentration occur. In particular, we show quantitatively that interference effects cause the turbidity to decrease as concentration increases beyond a certain volume fraction. These interference effects are due to correlations in the particles' positions introduced by the hardcore interactions between them. To make this calculation feasible, we assume, as a first approximation, that the particles are spheres of uniform size, and distributed throughout the volume of space not excluded by the presence of other spheres. Methods are available for calculating such distributions and their scattering properties (Ailawadi, 1980). Here we use the well-known Percus-Yevick approximation rather than that of Eq. 2. Because the refractive indexes of the dispersed particles, n_1, and the dispersing medium, n_2, are constant and because the spheres are of uniform size, only the proportions of the two phases will change.

We want to obtain the light scattering intensities in Rayleigh rations, R_θ

$$R_\theta = \frac{I_\theta r^2}{I_0 V} \quad (3)$$

where I_θ in the intensity of the scattered light at θ angle and I_o at zero scattering angle (incident light intensity), V is the scattering volume and r is the distance between the scatterer and detector.

For unpolarized light the differential scattering cross section $\sigma_{i(\theta)}$ for a small isolated spherical scatterer is given by Kerker (1969).

$$\sigma_{i(\theta)} = \frac{8\pi^4 a^6 n_2^4}{r^2 \lambda_0^4}\left(\frac{m^2 - 1}{m^2 + 2}\right)^2 (1 + \cos^2\theta). \quad (4)$$

In the Born approximation (Kerker, 1969) the scattered-light intensity at an angle θ by N spheres in a unit volume is given as

$$I_\theta = I_0(N/V)\, V \sigma_{i(\theta)}\, S(q) \quad (5)$$

where $S(q)$ is the structure factor, N/V is ρ, the density of the system (number of spheres per unit volume).

Combining Eqs. 3–5, we get 6.

$$R_\theta = \frac{8\pi^4 n_2^4 a^6}{\lambda_0^4}\left(\frac{m^2 - 1}{m^2 + 2}\right)^2 (N/V) S(q)(1 + \cos^2\theta) \quad (6)$$

where λ_o is the wavelength in vacuum, a is the radius of the sphere, n_1 and n_2 are the refractive indices of the sphere and the surrounding medium, respectively, and m is the ratio of the refractive indices:

$$m = \frac{n_1}{n_2}. \quad (7)$$

We give the density in terms of volume fraction of the spheres, ϕ,

$$\phi = (NV_s)/V \quad (8)$$

where V_s the volume of the sphere.

$$V_s = 4/3\, \pi a^3. \quad (9)$$

Substituting Eqs. 8 and 9 into Eq. 6 we obtain the Rayleigh ratio as a function of volume fraction.

$$R = \frac{6\pi^3 n_2^4 a^3}{\lambda^4}\left(\frac{m^2 - 1}{m^2 + 2}\right)^2 S(q)(1 + \cos^2\theta)\phi. \quad (10)$$

Because our particle sizes are comparable with the wavelength of the light, we must use the Rayleigh-Gans-Debye scattering theory and introduce the form factor $P(\theta)$ (Kerker, 1969).

For spheres this is

$$P_{(\theta)} = \left[\frac{3}{u^3}(\sin u - u\cos u)\right]^2 \quad (11)$$

where

$$u = (4\pi\, n_2/\lambda_0)\, a \sin\theta/2. \quad (12)$$

Therefore, the Rayleigh ratio for large spheres will be given

$$R_\theta = \frac{6\pi^3 a^3 n_2^4}{\lambda_0^4}\left(\frac{m^2 - 1}{m^2 + 2}\right)^2 (1 + \cos^2\theta)\, S(q) P(\theta)\phi. \quad (13)$$

Ailawadi (1980) has shown that the structure factor $S(q)$ is related to the Fourier transform $\tilde{c}(q)$ of the direct correlation function $c(r)$ by the expression

$$S(q) = 1/[1 - \rho\,\tilde{c}(q)]. \quad (14)$$

The function $\tilde{c}(q)$ depends on the volume fraction, ϕ, the size of the sphere, a, and the scattering angle, θ. In the Percus-Yevick approximation (Ailawadi, 1980, Eqs. 3.17 and 3.18) it is

$$\begin{aligned}\rho\tilde{c}(q) = {} & \\ & -\frac{24\phi}{(2ha)^6}\,(\alpha(2ha)^3[\sin(2ha) - 2ha\cos(2ha)] \\ & + \beta(2ha)^2[4ha\sin 2ha - (4h^2a^2 - 2)\cos 2ha - 2] \\ & + \gamma[(32h^3a^3 - 48ha)\sin 2ha - (16\, h^4a^4 - 48h^2a^2 + 24) \\ & \cdot x\cos 2ha + 24]\end{aligned} \quad (15)$$

where

$$h = \frac{4\pi n_2}{\lambda_0}\sin\theta/2. \quad (16)$$

The coefficients α, β, and γ are functions of the volume fraction of hard spheres, ϕ.

$$\alpha = (1 + 2\phi)^2/(1 - \phi)^4 \quad (17)$$

$$\beta = -6\phi(1 + \tfrac{1}{2}\phi)^2/(1 - \phi)^4 \quad (18)$$

$$\gamma = \frac{1}{2}\phi(1 + 2\phi)^2/(1 - \phi)^4. \quad (19)$$

Therefore the Rayleigh ratio of Eq. 13 at a certain angle (10°) can be calculated as a function of concentration (volume fraction) if the radius of the sphere and the refractive index of the sphere and medium are given.

RESULTS

Figs. 1 and 2 show the variation of the Rayleigh ratio at 10° for unpolarized light with concentration. Two diameters of model spheres were selected. The 100 nm diameter represents the average size of aggregates one finds in young human lenses in the cortical region that has the greatest transparency (Siew et al., 1981 *a*; Bettelheim et al., 1981 *b*). The 300 nm diameter corresponds to the average size of particles we obtained from light-scattering measurements on cataractous lenses (Siew et al., 1981 *b*; Bettelheim et al., 1981 *a*). In this and in all the subsequent data presentation, calculations were carried out only up to 0.4–0.5 volume fraction concentration. This was done because this is the biological range of interest, i.e., the average water content of the lens is 65%. Out of this, 76% is freezable (free) water in normal lenses, and 87% in cataractous lenses (Racz et al., 1979).

We assumed refractive indexes for the spherical aggregates between 1.68 and 1.50. This is the maximum range of refractive indexes in nuclear cataracts (Philipson, 1969, 1973). The range of refractive indexes selected for the embedding medium is also an extreme, 1.34–1.36. This again is indicative of a nuclear cataract in which syneresis took place and diluted the medium (Bettelheim, 1979).

Similar calculations were done on the concentration dependence of R_θ by using refractive index values that are likely to be found in normal lenses. For example, $n_1 = 1.46$; $n_2 = 1.43$; and $n_1 = 1.48$; $n_2 = 1.38$. These values yield

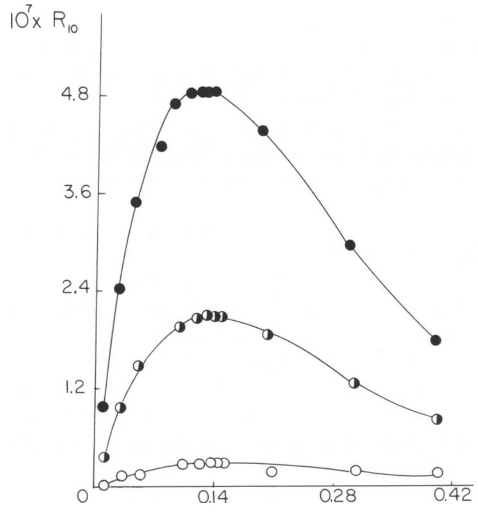

FIGURE 1 Calculated scattering intensities (Raleigh ratios at 10° scattering angle) in reciprocal nanometers as a function of volume fraction for randomly dispersed spheres of 100 nm diameter. $\lambda = 546$ nm. (●) $n_1 = 1.58$; $n_2 = 1.43$. (◑) $n_1 = 1.48$; $n_2 = 1.38$. (○) $n_1 = 1.46$; $n_2 = 1.43$.

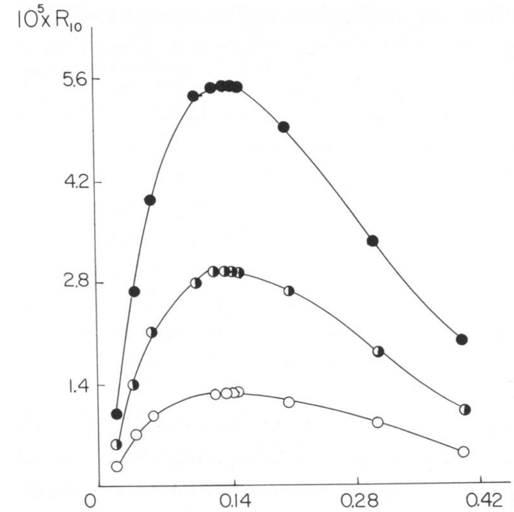

FIGURE 2 Calculated scattering intensities (Rayleigh ration at 10° scattering angle) in nm^{-1} as a function of volume fraction of randomly dispersed spheres of 300 nm diameter. $\lambda = 546$ nm. (●) $n_1 = 1.68$; $n_2 = 1.34$. (◑) $n_1 = 1.60$; $n_2 = 1.36$. (○) $n_1 = 1.50$; $n_2 = 1.34$.

amplitude factors that were obtained in normal lenses. In Fig. 1 parameters approximating normal lenses were used, namely 100-nm sphere diameter with a small refractive index difference between the spheres and the surrounding. It is interesting to note that the difference among the curves is only in the intensity of the scattered light. Therefore, a threefold increase in refractive index difference $(n_1 - n_2)$ gives a 10-fold increase in light intensity. Especially noteworthy is the behavior of light-scattering intensity with concentration. In dilute and moderately concentrated solution, up to 0.13 volume fraction, the light-scattering intensity increases with concentration. Above this concentration range, light-scattering intensity decreases.

The same trend can be observed in Fig. 2 in which light-scattering intensities were generated approximating the conditions in cataractous lenses. Again, the light-scattering intensity increases with concentration up to ~0.13 volume fraction after which it decreases. One can see that the cataractous condition gives a one- or two-magnitude greater amount of scattering than the normal condition. Furthermore, the size parameter alone going from 100 to 300 nm diameter of spheres increases the intensity of scattered light ~30-fold.

DISCUSSION

In the past we have shown that the random fluctuation theory of light scattering of Debye and Bueche (1949) can be applied to nuclear cataract in which syneresis and aggregation take place. In those processes the concentration of scattering elements increases (Bettelheim et al., 1981*a*).

The purpose of this study was to establish whether the random fluctuation theory can also account for the osmotic pressure–generated cataract formation (Kinoshita, 1974). In cataracts of the different etiology such as sugar (Kinoshita, 1965; Bettelheim and Bettelheim, 1978), ionizing radiation (Lambert and Kinoshita, 1967), and some hereditary cataract formation (Iwata and Kinoshita, 1971), an influx of Na^+ and Cl^- ions into the lens generates an osmotic pressure gradient that results in increased hydration of the lens. In extreme cases this excess water forms pockets of vacuoles and larger lakes. Even before such phase separation takes place, the turbidity increases as the concentration of protein and other solids decrease in the lens.

The dilute solution approximation predicts a decrease of turbidity with dilution. We have shown here that the full random fluctuation theory predicts an increase in turbidity upon dilution of the cytoplasmic gel, provided the starting volume fraction is greater than ~0.13. Such an increase is, in fact, observed in sugar cataract formation. In our previous investigations we have shown that the light-scattering properties of normal (Bettelheim and Paunovic, 1979) and nuclear cataract lenses (Bettelheim et al., 1981 *a*) can also be accounted for by the theory of random fluctuations (Debye and Bueche, 1949).

The description of cataract formation due to "spatial fluctuation of the refractive index formed by interspersed regions" (Ishimoto et al., 1979) is equivalent to our statements on the fluctuation of refractive index due to density and orientation fluctuations (Bettelheim and Paunovic, 1979). The difference is in the emphasis on reversible or irreversible processes. The designation of this phenomenon as a phase separation assumes the temperature-dependent reversibility of the opacification process. This is applicable to the cold cataract formation as well as to the nuclear cataract formation in galactosemic rat lenses (Ishimoto et al., 1979), but not to the cortical cataracts due to vacuole formation in the same galactosemic rats. The latter develops before the nuclear cataract formation and is the one that is truly associated with the osmotic pressure–generated cataractogenesis. In the present study, we have shown (Fig. 1 and 2) that the random fluctuation theory would also predict the development of turbidity in cataracts caused by osmotic pressure–generated hydration. If we assume a volume fraction of scattering units of ~0.3, for normal lenses, we can perceive a 50% increase in turbidity caused only by the dilution effect of influx of water. This corresponds to a 14% swelling (going from 0.7 to 0.8 volume fraction of water).

Beyond this dilution effect there are other changes not accounted for here in our simplified model. For one, we did not allow any swelling of the spheres, which would have increased their diameter as well as lowering their refractive index. These two changes, not contemplated in our model, act against each other and may cancel each other, thus supporting this model. We also neglect turbidity increase due to changes in optical anisotropy (Bettelheim, 1975; Bettelheim, 1978) upon swelling. This is a minor but important contributor especially, at the beginning of swelling, but it can be considered quantitatively only if one uses polarized light (Stein et al., 1959).

In conclusion, the random fluctuation theory provides a description that is in agreement with observed behavior both in the dilute solute range as well in gels or concentrated solutions. It explains equally the increase in turbidity in cataract formation due to aggregation, syneresis, and osmotic pressure-generated dilution effect. It covers the important concentration range of biological tissues, and therefore, the application of this theory renders the approximations of an infinitely dilute solution model or an ordered solid-state model unnecessary.

We would like to give thanks to R. A. Farrell and R. L. McCally of the Johns Hopkins University Applied Physics Laboratory for calling to our attention the methods of hard-sphere modeling and for the helpful discussion of some of the computation problems.

This research was supported by a National Eye Institute grant EY-02571-03.

Received for publication 12 September 1980 and in revised form 15 September 1982.

REFERENCES

Ailawadi, N. K. 1980. Equilibrium studies of simple liquids. *Phys. Reports* (*Review Section of Physics Letters*). 57C 4:241.

Benedek, G. M. 1971. Theory of transparency of the eye. *Appl. Opt.* 10:459.

Bettelheim, F. A. 1975. On the optical anisotropy of lens fiber cells. *Exp. Eye Res.* 21:231.

Bettelheim, F. A. 1978. Induced optical anisotropy fluctuation in the lens of the eye. *J. Colloid Interface Sci.* 63:251.

Bettelheim, F. A., and A. A. Bettelheim. 1978. Small angle light scattering studies on xylose cataract formation in bovine lenses. *Invest. Ophthalmol. Visual Sci.* 17:896.

Bettelheim, F. A. 1979. Syneresis and its possible role in cataractogenesis. *Exp. Eye Res.* 28:189.

Bettelheim, F. A., and M. Paunovic. 1979. Light scattering of normal human lens. I. Application of random density and orientation fluctuation theory. *Biophys. J.* 26:85–99.

Bettelheim, F. A., E. L. Siew, and L. T. Chylack, Jr. 1981 *a*. Studies on human cataracts. III. Structural elements in nuclear cataracts and their contribution to turbidity. *Invest. Ophthalmol. Visual Sci.* 20:348.

Bettelheim, F. A., and E. L. Siew. 1980. Light scattering and lens morphology. *Dev. Biochem.* 9:443.

Bettelheim, F. A., E. L. Siew, S. Shyne, P. Fransworth, and P. Burke. 1981 *b*. A comparative study of human lens by light scattering and scanning electron microscopy. *Exp. Eye Res.* 32:125.

Chylack, Jr., L. T., F. A. Bettelheim, and W. H. Tung. 1981. Studies on human cataract. I. Evaluation of techniques of human cataract preservation after extraction. *Invets. Ophthalmol. Visual Sci.* 20:327.

Debye, P., and A. M. Bueche. 1949. Scattering by an inhomogeneous solid. *J. Appl. Phys.* 20:518.

Hoenders, H. J., and G. J. Van Kamp. 1972. Eye lens development and aging processes of crystallins. *Acta Morphol. Neerl. Scand.* 10:215.

Ishimoto, C. P., W. Goalwin, S. T. Sun, I. Nishio, and T. Tanaka. 1979. Cytoplasmic phase separation in formation of galactosemic cataract in lens of young rats. *Proc. Natl. Acad. Sci. U. S. A.* 76:4414.

Iwata, S., and J. H. Kinoshita. 1971. Mechanism of development of heredity cataract in mice. *Invest. Ophthalmol.* 10:504.

Jedziniak, J., J. H. Kinoshita, E. M. Yeats, and G. Benedek. 1975. The concentration and localization of heavy molecular weight aggregates in aging normal and cataractous human lenses. *Exp. Eye Res.* 20:367.

Jedziniak, J., D. F. Nicoli, H. Baram, and G. Benedek. 1978. Quantitative verification of the existance of high molecular weight protein aggregates in the intact normal human lens by light scattering spectroscopy. *Invest. Ophthalmol. Visual Sci.* 17:51.

Kerker, M. 1969. The Scattering of Light and Other Electromagnetic Radiation. Academic Press, Inc., New York. 30–40.

Kinoshita, J. H. 1965. Cataracts in galactosemia. *Invest. Ophthalmol.* 41:786.

Kinoshita, J. H. 1974. Mechanism initiating cataract formation. *Invest. Ophthalmol.* 13:713.

Lambert, B. W., and J. H. Kinoshita. 1967. The effect of ionizing radiation on lens cation permeability, transport, and hydration. *Invest. Ophthalmol.* 6:625.

Philipson, B. 1969. Light scattering in lenses with experimental cataracts. *Acta Ophthalmol. Suppl.* 47:103.

Philipson, B. 1973. Changes in the lens related to the reduction of transparency. *Exp. Eye Res.* 16:29.

Racz, P., K. Tompa, and I. Pocsik. 1979. The state of water in normal and senile cataractous lenses studies by nuclear magnetic resonance. *Exp. Eye Res.* 28:129.

Siew, E. L., D. Opalecky, and F. A. Bettelheim. 1981 *a*. Light scattering of normal human lens. II. Age dependence of light scattering parameters. *Exp. Eye Res.* 33:603–614.

Siew, E. L., F. A. Bettelheim, L. T. Chylack, Jr., and W. H. Tung. 1981 *b*. Studies on human cataract. II. Correlation between the clinical description and the light scattering parameters of human cataracts. *Invest. Ophthalmol. Visual Sci.* 20:334.

Siezen, R. J., and H. Berger. 1978. Quaternary structure of bovine α-crystallin. *Eur. J. Biochem.* 91:397.

Spector, A. 1972. Aggregation of α-crystallin and its possible relationship to cataract formation. *Isr. J. Med. Sci.* 8:1577.

Stein, R. S., J. J. Keane, F. H. Norris, F. A. Bettelheim, and P. R. Willson. 1959. Some light scattering studies of the texture of crystalline polymers. *Ann. N. Y. Acad. Sci.* 83:37.

Trokel, S. 1962. The physical basis of transparency of crystalline lens. *Invest. Ophthalmol.* 1:493.