DETERMINATION OF THE PARAMETERS OF LIPOSOMES BY THE TURBIDITY SPECTRUM METHOD

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There has been a marked increase in recent years in the number of investigations into different aspects of the use of liposomes — spherical phospholipid vesicles — in medicine. This is particularly true of the creation of microencapsulated forms of drugs and, in particular, of microcapsules with high- and low-molecular-weight compounds. An important problem connected with the use of liposomes is the development of simple and readily available methods of determination of the size of microcapsules and detection of any changes taking place in them during keeping. Rapid and continuous measurements of the parameters of liposomes would allow a check to be kept on processes involved in their preparation and a study of the distribution of these vesicles, depending on their size, among the organs and tissues of experimental animals.

Usually the dimensions of liposomes are determined from photomicrographs [9]. More recently methods of estimation of the size of liposomes by gel filtration [11] and electrophoresis [14] have been suggested. Many investigators also judge changes in the state of liposomes as reflected in the optical density of a liposome suspension (D), measured at a fixed wavelength (λ). An increase in D (a decrease in light transmittance — T) at a given λ may in fact be evidence of aggregation of liposomes and an increase in their size, such as on the addition of a certain agent [8, 12] or with a rise of temperature [5]. However, it must be noted that a change in D, not at a certain value of λ but within a range of wavelengths, when substances contained in liposomes do not absorb (for example, in the 400-600 nm region), enables the mean diameter of the scattering particles (d_λ) and their number per unit volume (N) to be estimated by the turbidity spectrum method. This method has been developed in detail by Klenin et al. for supramolecular particles and cells in a number of publications [1, 2].

EXPERIMENTAL METHOD

The basic idea of the method is that the smooth character of the turbidity spectrum $\tau = \tau(\lambda)$ within a narrow interval $\Delta\lambda$ enables the turbidity spectrum to be approximated by an Angstrom relationship: $\tau \sim \lambda^{-n}$, where the turbidity τ for a nonabsorbing scattering system is related to D by the formula: $\tau = 2.3D/l$, where l is the length of the optical path, and where n, the wave exponent, can be determined from a graph of log D versus log λ: $n_\lambda = -\Delta\log D/\Delta\log\lambda$. The wave exponent is a dimensionless function of two parameters $n = n(\alpha, m)$, where $m = m_1/m_0$ is the relative refractive index (m_1 and m_0 are the refractive indices of the particles and medium, respectively), and α is related to the diameter of the particles d: $\alpha = \pi d m_0/\lambda$. The values of $n(\alpha, m)$ are given in Tables for $\alpha > 1$ in [1] and for $\alpha < 1$ in [7]. The relative refractive index of liposomes can be determined by a photometric method [3, 4]. For liposomes of varied composition which were used in the present experiments, the values of m lie in the interval 1.03-1.09.

EXPERIMENTAL RESULTS

A graph of the wave exponent (n) as a function of particle diameter (d), plotted from data in tables [1, 7] for λ = 500 nm, m_0 = 1.33 (physiological saline), and m = 1.03, 1.05, and 1.07, characteristic of liposomes. It must be noted that if $d \leq 0.5$ μ (Fig. 1B), the particle size can be determined from n without the need to determine m [1]. Indeterminacy

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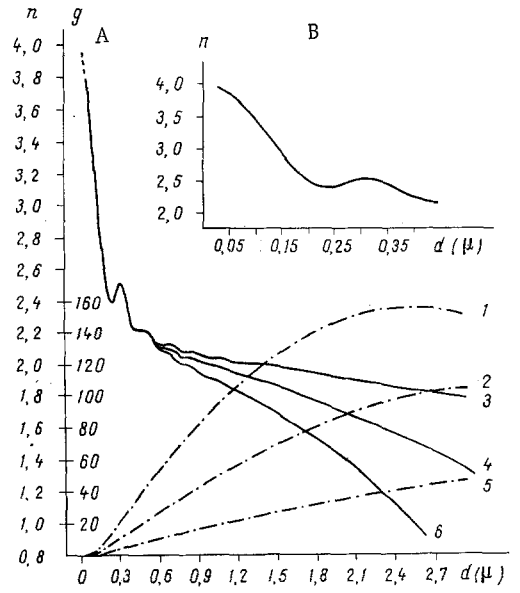

Fig. 1

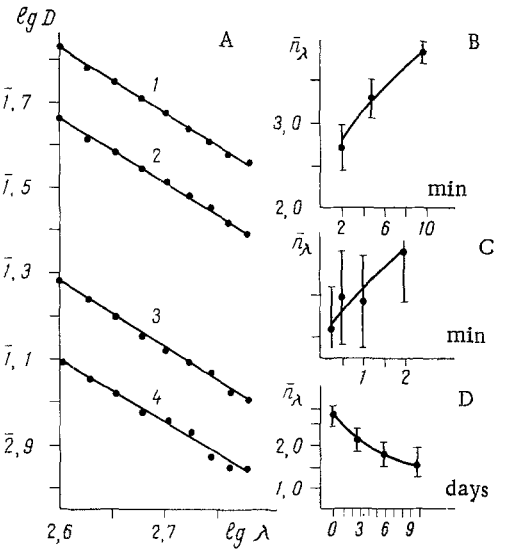

Fig. 2

Fig. 1. Wave exponent n (3, 4, 6) and specific turbidity g (1, 2, 5) as functions of diameter of scattering particles d. A) λ = 500 nm; m_0 = 1.33; l = 1 cm; m = 1.03 (3, 5), 1.05 (2, 4), and 1.07 (1, 6). Values for g given in cm^{-1}, for d in μ. B) n as a function of d when d $\leq$ 0.5 μ.

Fig. 2. Log D as a function of log λ for suspensions of liposomes with different phospholipid concentration (A): n = 1.54 (1), 1.52 (2), 1.57 (3), and 1.48 (4), and wave exponent n as a function of duration of exposure of liposome suspension to ultrasound (B, C) and as a function of its keeping at room temperature (D). Liposome suspensions prepared by methods described in [6, 10] (B, D) and in [13] (C).

at n = 2.4-2.5, if significant, can be avoided by increasing the time of treatment of the liposome suspension, with ultrasound for example, by very little. Turbidity spectra of various dilutions of a suspension of liposomes of phosphatidylcholine in physiological saline, prepared by the method in [13], are given on a double logarithmic scale in Fig. 2A. The spectra were recorded on the SF-16 spectrophotometer at points every 25 nm within the interval 400-600 nm. To reduce the chances of scattered light falling on the photoelectric cell, diaphragms were placed in front of the entry slit of the instrument and in front of the photoelectric cell. It will be clear from Fig. 2A that the wave exponent was independent of particle concentration, and this is one of the main advantages of the method. Meanwhile measurement of D at a fixed value of λ allows the concentration of liposomes to be determined if the parameters α and m [1] are known. For λ = 500 nm (the middle of the interval measured), m_0 = 1.33, and l = 1 cm, the number of particles per unit volume is determined by the equation:

$$N = 4 \cdot 10^{10} \frac{D_{500}}{g(\alpha, m) \cdot d^3} \ [\text{cm}^{-3}],$$

where D_{500} is the optical density at 500 nm (for suspensions not absorbing in this region); d is the particle diameter (in μ); $g(\alpha, m)$ the specific turbidity — a characteristic function of scatter of light, given in tables for $\alpha > 1$ in [1].

From data in these tables, dependence of g on d for λ = 500 nm, m_0 = 1.33, l = 1 cm, and m = 1.03, 1.05, and 1.07, is given in Fig. 1. The particle density can be determined from the specific turbidity [1].

The turbidity spectrum method can thus be used to characterize suspensions of liposomes with particle diameter ranging approximately from 0.05 μ to several microns quickly, without the need to subject the suspension to additional procedures. The error of determination of the mean diameter $\bar{d}_\lambda$ depends on the degree of dispersion of the liposome suspension [1] and in practice it is 5-10% for distribution of liposomes by size of the type described in [9]. Examples of a change in wave exponent ($\bar{n}_\lambda$) for liposomes of different composition and the method of preparation for treatment with ultrasound under different conditions and during

keeping, are given in Fig. 2. It will be clear from Fig. 2B that with an increase in the duration of ultrasound treatment the mean diameter of the liposomes fell from 0.17 to 0.06 μ (determined from Fig. 1B), whereas during keeping (Fig. 2D) $\bar{d}_\lambda$ increased from 0.2 μ to approximately 2 μ, for on the 10th day of keeping at room temperature the relative refractive index of these liposomes was 1.06 ± 0.01. By separating the suspension into fractions, by gel-filtration for example, the distribution of liposomes by size can be obtained. The turbidity spectrum method can also be used to analyze fractions of liposomes after ultracentrifugation, to monitor the process of preparation of liposomes, and to verify their state after exposure to various factors.

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DETERMINATION OF THE PROBABILITY OF AGGREGATION TO ASSESS THE FUNCTIONAL STATE OF PLATELETS

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Investigations of platelet aggregation induced by addition of ADP, serotonin (or other factors) in platelet-enriched blood plasma provide information on the functional state of the platelets and are widely used at the present time. The turbidimetric method, developed by Born [2], is used for this purpose.

However, there is as yet no satisfactory approach to the interpretation of results obtained by Born's method which would allow quantitative evaluation of the state of platelets on the basis of analysis of a series of aggregatograms; moreover, it is difficult to compare aggregatograms obtained for different samples of blood plasma.

A method of determining the probability of platelet aggregation due to collision at the beginning of the aggregation process, based on analysis of the initial portion of the aggretogram, is suggested in this paper. The parameters usually obtained by analysis of aggregatograms (a change in light transmittance of the cell suspension at the maximum of aggregation, the rate of change of light transmittance during aggregation and disaggregation) depend es-

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