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INVERSE PROBLEMS OF SPECTROTURBIDIMETRY OF BIOLOGICAL DISPERSE SYSTEMS: AN OVERVIEW

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ABSTRACT

The obtainment of stable solutions of inverse problems for studying the disperse composition of suspensions using effects of elastic light scattering was discussed. Versions of a regularization of solving the inverse problems of the spectroturbidimetric method were considered, taking into account unavoidable restrictions on the scope of the necessary prior data for particles and on the width of the spectral interval for real biological disperse systems. Possibilities for increasing the number of particle parameters determined in a single optical experiment were analyzed. They were shown to be provided by the use of effects of the orientation ordering of a system on combination of capabilities of the methods of spectroturbidimetry and electro-optics, using bacterial cell suspensions as an example. [S1083-3668(99)00304-4]

Keywords light scattering; disperse systems; inverse problems; regularization; spectroturbidimetry; electro-optics.

1 INTRODUCTION

The prevalence of disperse systems in nature, and their great significance to medicine, biological, chemical, space, and other technologies have stimulated the development of various methods for quantitative analysis of disperse systems. Among these methods are those based on the *elastic* light scattering effects.[1–8] For this type of interaction between a substance and light (unlike, for example, the quasielastic or dynamic light scattering)[9–11] there is no energy exchange between a light quantum and a particle. Therefore, the wave frequency remains the same during the scattering process.

Among many kinds of disperse systems consisting of biological or synthetic macromolecules, of considerable significance are the so-called *ill-defined* complex systems. They are distinguished by a difficulty in obtaining all the necessary *preliminary* information on particle structure, form, concentration, and composition of a dispersion medium. Generally, one needs information of this kind while interpreting data from optical measurements on the basis of one or another of the physical models which include a set of system parameters. It hinders essentially the potentialities of many well-recognized methods since most of them are usually not self-sufficient (some exceptions to this rule will be given in this paper). Examples of such systems are suspensions of supramolecular particles in solutions and gels of bio- and synthetic polymers (including biotissues), subcellular particles, insoluble immune complexes, suspensions of some microorganisms and viruses, human and animal blood cells, etc.

In connection with problems of simulation of optical effects in disperse systems and quantitative interpretation of light scattering data, direct and inverse problems are recognized.[5,12–15] By the *direct problem*, one means the study of light scattering characteristics when those of a substance and a light beam incident on it are specified. By the *inverse problem*, one means the determination of characteristics of the substance volume element where light is scattered (specifically, particle size and concentration) from measured characteristics of the incident and scattered radiation. Unlike the direct problems, the inverse problems are far from always being solvable since the detectable scattered radiation may lack information about the investigated substance's properties. Moreover, even if a single solution formally exists, it may appear to be practically inconclusive due to the catastrophic effect of unavoidable experimental errors on the final outcomes.[5,13] This results from the basic mathematical incorrectness of a great number of inverse physical problems of probing a substance by various types of radiation. In connection with this, the

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stability of a method should be recognized as one of the main indices characterizing the quality of any method of the inverse problem solution. Relationships between the errors of measurements of the initial scattering characteristics and the errors of determining the desired disperse system's parameters may serve as a measure of the stability. Coefficients of amplification of errors are its quantitative characteristics.[5]

In order to illustrate some consequences of the incorrectness, one can use a basic relationship for the normalized numerical particle size distribution function $f(r)$:[5]

$$T(\theta,\lambda,\mu,\mu_0)=N\int_{r_{\min}}^{r_{\max}} t(r,\theta,\lambda,\mu,\mu_0)f(r)dr, \quad (1)$$

where θ is the scattering angle, λ is the light wavelength in vacuum, N is the number of particles per unit of suspension volume, μ and μ_0 are the refractive indices of particles and of a dispersion medium, respectively. It is assumed, for definiteness, that suspended particles are homogeneous spheres (r is the particle radius) and their concentration is small enough for a *single scattering approximation* to be applied. In the left part of this equation, one can use any measurable light scattering characteristic. It may be, e.g., an angular dependence of the scattered light intensity (indicatrix), a wavelength dependence of turbidity, etc. This expression is the Fredholm linear integral equation of the first kind for the desired distribution function. Its kernel t is the corresponding solution of the problem in the case of a rigorously monodisperse system of particles of radius r. The problem of inverting Eq. (1) consists of developing an algorithm of $f(r)$ determination with the previously chosen kernel and measured left part of the equation.

However, for such a general formulation, the solution of the problem involves difficulties resulting from the mathematical incorrectness of problems of inverting integral equations of the first kind.[5,12–15] To make it clear, note that in a number of cases, the fixed integrating limits $0\rightarrow\infty$ and kernels of the exponential type can be used. Inverse problems of this type appear, for instance, in various versions of the method of quasielastic light scattering[9–11] and in an electro-optical analysis of suspensions when inverting curves of orientational relaxation.[16–19] The latter are determined by the Brownian rotary diffusion of particles and depend on their optical properties.[20,21] The problem of inverting can then be reduced to the use of Fourier transformation, which allows one to make a simple illustration of the consequences of the incorrectness of integral equations [Eq. (1)].[5] Indeed, replacement solution $f(r)$ with a function

$$f^{*}(r)=f(r)+A\exp(i\omega r), \quad (2)$$

where A and ω are arbitrary parameters, changes the left part of the equation by the value

$$\Delta T=AN\int_0^{\infty} t(r,\theta,\lambda,\mu,\mu_0)\exp(i\omega r)dr. \quad (3)$$

If the frequency ω is high enough, the Fourier component ΔT of the function t may become so small that it falls within the interval of errors of experimental determination of T. Therefore, both functions $f(r)$ and $f^{*}(r)$ will practically satisfy the initial integral equation, even though if constants A and ω are chosen properly, these functions may significantly differ from each other. Thus, the unavoidable errors of measuring T limit the number of high harmonics, which can be recovered by solving the integral equation considered.

In the period from the 1940s to the 1960s, rigorous mathematical approaches to the solution of incorrect problems were developed. Note that such problems are widespread in various fields of physics and technology. Thorough studies in this field have been presented in books by Tikhonov and Arsenin[14] and Twomey.[15] Overcoming the mathematical incorrectness permitting a stable algorithm for the solution of an inverse problem to be developed is called *regularization*.[5,13,14] It is based on the idea of involving additional information on the system being studied (i.e., imposing some reasonable restrictions on it) which makes the problem correct. This essentially results in the appearance of a *new* problem whose solution must satisfy the following conditions. *First*, it has to be close enough to the solution of the initial physical problem and *second*, it must be stable. At present, there exist rigorously formalized general procedures of regularization.[14]

2 REGULARIZATION IN SOLVING THE INVERSE PROBLEMS

From the 1950s, pioneering studies on some inverse problems of the light scattering method have been carried out by the research school headed by Shifrin. Some of their general conclusions can be found in Refs. 5 and 22. Among books written in English, the collected articles edited by Baltes[12] dealing with theoretical aspects of various inverse problems in physical optics could be mentioned as an example.

2.1 INVERSE PROBLEMS OF THE LIGHT SCATTERING METHOD

The variants of rigorous analytical solutions of the above mentioned integral equation for a number of important special cases[5,22] are of great theoretical and practical interest. In these cases, the equation kernel is expressed by relatively simple formulas. These variants were called the small angles method, the complete indicatrix method, and the spectral transparency method. According to Shifrin,[5] regularization in these methods is achieved by the suggestion that all improper integrals that are necessary for their realization really exist.

The *small angle method* uses an angle distribution of intensity of light diffracted on large particles near the direction of light propagation from a source of radiation (halo). The problem is reduced to inverting the integral equation

$$I(\theta)=\frac{I_0}{\theta^2}\int_0^\infty f(r)r^2J_1^2\left(\frac{2\pi\mu_0}{\lambda}\theta r\right)dr, \tag{4}$$

where I_0 is the incident light intensity, $I(\theta)$ is an experimental angle distribution, and J_1 is the Bessel function of the first order. In this case, the following relationships should be fulfilled:

$$\alpha=2\pi r\mu_0/\lambda\gg 1,\quad \theta\ll 1,\quad \alpha\theta>0. \tag{5}$$

The solution of Eq. (4) is

$$f(\alpha)=-\frac{4\pi^2}{r^2\lambda}\int_0^\infty F(\alpha\theta)\frac{d}{d\theta}[\theta^3I(\theta)/I_0]d\theta, \tag{6}$$

$$F(x)=xJ_1(x)Y_1(x), \tag{7}$$

where Y is the Neumann function of the first order. Extensive literature exists on the peculiarities of the experimental realization of the method, which involves requirements for the angle range containing all the necessary optical information (see reviews of Refs. 5 and 22). The range is from approximately 10 min to 10°. It is an obvious advantage of this method that it requires no knowledge of the particle refractive index. However, the refractive index defines the lower limit of the particle size range since the limitation by the value of a phase shift parameter ρ serves as one more condition of the method's applicability

$$\rho=2\alpha|m-1|\gg 1, \tag{8}$$

where $m=\mu/\mu_0$ is the relative particle refractive index. Resulting from this condition, e.g., for biological suspensions characterized by the relatively small values of $m\approx 1.03$–1.05, the small angle method is applicable only for large particles with diameters of a few tens of microns and higher.

The *complete indicatrix method* is analogous, in principle, to the previous approach. However, it has a lower limit of applicability by particle size of approximately an order of magnitude less. For a polydisperse system of optically soft nonabsorbing particles with a slight difference between the particle and medium refractive indices, the following equation is valid:

$$I(\theta)=\int_0^\infty I(\theta,r)f(r)dr, \tag{9}$$

where $I(\theta,r)$ denotes an analytical expression for the indicatrix of ideally monodisperse systems obtained in the Rayleigh–Gaus–Debye (RGD) approximation.[1,2] This approximation may be applicable in the following conditions:

$$|m-1|\ll 1,\quad \rho\ll 1. \tag{10}$$

The exact solution of this equation is

$$f(r/r_0)=\frac{1}{(r/r_0)^2}\int_0^\infty h(rx/r_0)u(x/2)dx, \tag{11}$$

where r_0 denotes some linear scale, usually equal to some mean or modal radius r_m, h involves only elementary functions, and u includes the experimental polydisperse indicatrix $I(\theta)$. As in all variants of inverting integral equations, it is very important to choose proper limits of integration (the range of angles where the indicatrix is measured). In this case, the photometric accuracy of up to 5% is sufficient, and the lower limit by the modal particle radius has the order of the wavelength used.[5] For suspensions with a smaller particle size, the indicatrix shape in the region of relatively large angles approaches the Rayleigh one, and it becomes practically impossible to determine the complete composition of a disperse system from it.

In the spectral transparency method (STM), the initial integral equation is as follows:

$$\tau(\varsigma)=\frac{\pi}{r_0}\int_0^\infty K(\rho)(r^*)^2f(r^*)dr^*, \tag{12}$$

where τ is the system's turbidity, r_0 is the above mentioned linear scale $\varsigma=1/\lambda$, and $r^*=r/r_0$. In the expression for the kernel of this equation, the *scattering coefficient* (efficiency factor) for monodisperse systems $K(\rho)$ is used, which was obtained by van de Hulst[1] under the first parts of conditions (5) and (10) [anomalous diffraction (AD) approximation]. Thus, large enough optically soft nonadsorbing particles with no restrictions on the phase shift parameter are meant. The solution of this equation has the following form:

$$f(r/r_0)=\frac{1}{\pi^2(r/r_0)}\int_0^\infty w(rx/r_0)q(x/2)dx, \tag{13}$$

where $x=4\pi r_0(m-1)\varsigma$, w is expressed by elementary functions only, and q includes the experimental dependence of turbidity on the wave number. The wavelength interval necessary for the stable recovery of the size distribution function is sure to involve that in the region of the *major diffraction maximum* $\tau_{\max}$. The regions of monotonous change in the wavelength dependence of turbidity do not contain enough information to correctly solve the complete inverse problem considered.

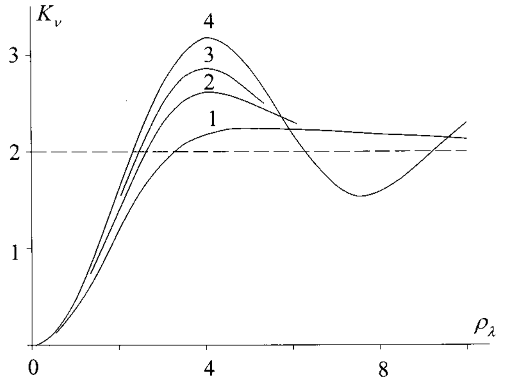

Fig. 1 Dependence of K_ν (Ref. 24) on r_λ (Ref. 23) at $\nu=0$ (1), 5 (2), 15 (3), and $\nu\to\infty$ (4).

It may be shown that $\tau_{\max}$ corresponds to the first maximum of the dependence of the efficiency factor K on ρ, where K is defined by the following expression:

$$K=\tau/S_0, \tag{14}$$

where

$$S_0=N\pi r_2^2 \tag{15}$$

is an effective total area of the maximum particle cross section, and r_2 is the particle mean-square radius. In this case, it is more convenient to use a special type of averaging r_λ[23] (instead of r_2) which coincides with a mean-surface radius r_S in the region of the major diffraction maximum. It permits a vivid comparison of the results of K calculations made to various degrees of the system's polydispersity due to the relatively weak dependence of the abscissa of $K_{\max}$ on the degree of polydispersity of a system. This is illustrated in Figure 1, which contains the dependences of K_ν on ρ_λ resulting from analytical calculations of K_ν[24] in the AD approximation made for the model gamma distributions

$$f(r)=\beta^{\nu+1}r^{\nu}\exp(-\beta r)/\Gamma(\nu+1), \tag{16}$$

where β and ν are independent parameters, and $\Gamma(x)$ is the gamma function. The value of $\nu=0$ gives the widest size distribution in this family, while the value of $\nu\to\infty$ means turning to monodisperse systems. Numerical experiments made on the basis of such calculations showed[5,22] that, with the error of turbidity measurements of up to 5%, satisfactory results can be obtained if spectroturbidimetric data in the range of the far ultraviolet to infrared region of the light spectrum are used. In this case, the smaller the particle size and refractive index, the lower the values of the wavelength that have to be used. For example, if $m=1.1$ and r_m is within the interval of 0.1–1 μm (typical values for many of polymer and biopolymer systems), λ varies from 60 to 2000 nm.

It is worth noting that, according to the data of many works (see, for instance, Refs. 1–5 and 25), the values of light scattering functions obtained in the approximation of *optically soft particles* agree satisfactorily with the results of the corresponding rigorous solutions (based on Mie's theory[1,2,6]) at least for $0.8\leqslant m\leqslant 1.5$. Furthermore, at values of $m \leqslant 1.15$, they in fact coincide on the level of small enough errors. RGD and AD approximations are mutually complementary and allow one to consider the highest possible range of values of the light scattering functions and particle parameters. Both of them are applicable in principle to particles of arbitrary shapes and have a common boundary at $\alpha\gg 1$ and $\rho\ll 1$, respectively. For *nonspherical* particles, the T-matrix method[6,26,27] developed for isotropic spheroids can be mentioned as an example of the modern approach to the rigorous solution of the electrodynamic problem. For modeling objects with a complex structure, in addition to known calculation schemes using patterns of layered spherical particles,[4,6,7,28,29] the method of connected dipoles[6,30] may probably have a good outlook.

To date, mathematical methods of regularization of solution of inverse physical problems, including standardized numerical procedures of their computer realization, have been developed. Methods of mathematical statistics[31] were used for a stable reconstruction of $f(r)$ using spectroturbidimetric[32] and nephelometric[33] data. In particular, the method of statistical regularization permits the above discussed method of complete indicatrix to be extended to particles with an *arbitrary* (including complex) value of the relative refractive index and to multimodal systems. Besides, it is noted in Ref. 5 that there is a possibility of estimating the value of the refractive index itself from the same nephelometric experiment using the dependence of stability of solutions obtained on the proper prior choice of the refractive index values.

In conclusion of this brief review of the variants of solution of the complete inverse problem for determining particle size distribution functions, we note the following. The small angle method and the method of the complete indicatrix, mutually supplementing each other, cover a wide range of particle sizes, which can be compared to the range of theoretical applicability of the STM. However, from the point of view of practical investigations of ill-defined systems, spectroturbidimetric analysis has significant advantages over nephelometry. *First*, special calculations[34] done on the basis of the radiation transfer theory showed the results of turbidimetry for typical experimental conditions to be weakly dependent on the multiple light scattering effects. Thus, they can be interpreted using the single scattering theory even at relatively high values of particle concentration when the multiple scattered radiation already makes an overwhelming contribution in the whole scattered light beam and in that scattered at small angles. *Second,* one has to be aware that real suspensions in a great number of cases involve particles with a complex internal structure (for example, cells) and rather uncertain morphological properties (for instance, new phase particles in systems of complex-forming macromolecules). Numerous literature data obtained for various models of structured and nonspherical particles[4,6,7,28,29,35–37] showed that turbidimetric (unlike nephelometric) results were affected mainly by particle volume and concentration, practically irrespective of internal structure details and shape of moderately anisometric particles with random orientation. All these facts give every reason to solve correctly the inverse problems of *spectroturbidimetry* in terms of the relatively small number of, so to say ''main,'' parameters of disperse systems: particle size, refractive index, and concentration.

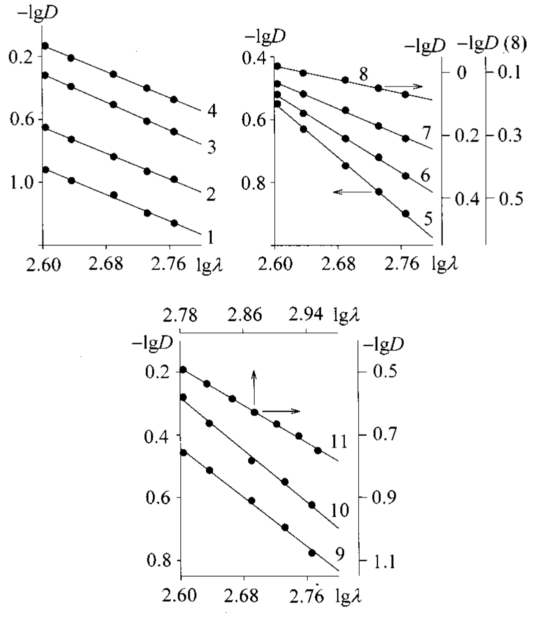

Fig. 2 Plots of log D vs log λ for various systems (Ref. 3).

2.2 TAKING ACCOUNT OF FEATURES OF REAL SYSTEMS IN THE SPECTROTURBIDIMETRIC ANALYSIS

Unfortunately, for a wide range of real biological disperse systems, it is rather difficult to recover the complete function of distribution in particle sizes by the STM. The effect of the strong absorption of light, for example, by proteins and nucleic acids in the ultraviolet region of light spectrum and cell pigments in its visible region (many synthetic polymers and organic solvents also absorb light in the ultraviolet) could in principle be overcome.[38] However, even in this case in order to reliably detect the main diffraction maximum and find the necessary short-wave asymptotic of the nonmonotonous spectral turbidity curve,[5,22] the spectral interval required seems to be too wide (from an instrumental standpoint) for particles with a characteristic linear size of several microns and smaller (see above). Figure 2 gives some characteristic experimental data obtained for a number of disperse systems:[3] acrylate latex in a series of dilutions (1–4), suspensions of polystyrene precipitated from its benzole solution by different quantities of methanol (5–8), a cell suspension of *Staphylococcus aureus* (9), a suspension of supramolecular particles of a 2% water solution of polyvinylalcohol (10), and products of reaction of rabbit antibodies with human immunoglobulins (11). These examples show that, in representative transparency ''windows'' being recorded with the use of routine spectroscopy equipment, whose width does not exceed approximately 500 nm, the turbidity $\tau = 2.3D/l$ (where D is the optical density and l is the cell length) usually exhibits itself as a practically monotonous power function of the wavelength. That is why recovery of the complete function of particle sizes distribution in these cases is practically impossible due to the forced lack of the necessary optical information and, as a result, sharply increased instability of formal solutions of the inverse problem.[5]

The increase in stability is achieved by procedures which may be called reasonable compromise tactics. In particular, sufficient stability of algorithms of inversion of spectroturbidimetric data in the *narrow* wavelength interval is achieved by limiting the problem to correct determination of integral parameters,[3,23,29,39] such as the mean particle size (or the radius of the equivalent volume sphere), the numerical and mass-volume concentration of a suspension, and the particle effective refractive index. A version of the spectroturbidimetric method (primarily developed for classical systems like latex) actually using such a compromise originated in the works of a group of American researchers led by Heller.[40–42] A Russian research team under the supervision of Klenin began to develop this method as applied to structurally complex systems.[3,23,29,43–47]

One of the main scattering characteristics used in this version of the spectroturbidimetric method is the wavelength exponent n in the following approximation expression for turbidity:

$$\tau = \text{const}\,\lambda^{-n}. \tag{17}$$

In experiments, n is expressed by way of the turbidity measured in a small enough spectral interval (about 200 nm) by the relationship

$$n = -\partial \ln \tau / \partial \ln \lambda. \tag{18}$$

Actually, n is an angular coefficient (taken with the inverse sign) of plots like those given in Figure 2. It is worth mentioning that it is independent of particle concentration. The substitution in Eq. (18) of the theoretical expression for τ obtained for some disperse system models results in equations for determination of either the particle size[40–42] or the particle refractive index.[43] In the first case, the particle refractive index has to be determined beforehand in an independent experiment. However, if particles are small enough ($r \leqslant 0.1$–$0.2\,\mu$m, $n > 2$), n is practically independent of m, and it becomes possible to determine the particle size without prior knowledge of m. In the second case, the same must be done for the particle size (for versions of spectroturbidimetry for simultaneous determination of mean particle size, refractive index, and concentration of latex suspensions, see Refs. 23 and 48). This method of determination of μ is effective for relatively large particles whose radius of the sphere of equivalent volume r is not less than about 0.5 μm ($n < 2$). In this case, light microscopy can be used for an independent determination of the particle size.[49] For particles that have a rigid enough surface (for example, yeast cells), one can use the immersion–photometric method for μ determination with a modification,[50] which extends its range of applicability in the particle size. In this case, a ρ dependence of n for large enough particles is taken into consideration.

As a first approximation, n can be calibrated by the formula

$$n(\alpha,m) = \partial \ln K(\alpha,m) / \partial \ln \alpha \tag{19}$$

with the scattering efficiency factor $K(\alpha,m)$ calculated for monodisperse systems of homogeneous spherical isotropic particles. In Eq. (19), the dispersion of optical constants of the particle and medium substances is not taken into consideration. The principal formulation of this problem can be found, for example, in Refs. 42 and 51. A summary of approach,[52] permitting dispersion to be taken into account in a wide range of experiments, is given below in this paper. Combining results of measurements of the wavelength exponent (particle size) and turbidity, one can determine (if m is known):[3,29,39,44–47,53] the number of particles per unit of suspension volume N, their mass–volume concentration C, and an effective total surface square S of particles occupying unit of suspension volume as well. The parameter C often has the meaning of concentration of the disperse phase pure substance (for example, of polymer precipitated as the result of phase separation,[29,46,47] immunochemical reaction,[54] etc.), or concentration of the dry intact cell substance (biomass concentration).[55] The parameter S can be used, for example, in experimental investigations of adsorption processes in disperse systems and for calculation of adsorption isotherms.[53,56] For this purpose, the following characteristic light scattering functions are used:

optical scattering cross section

$$\frac{\tau}{N} = \frac{(\lambda/\mu_0)^2}{4\pi}\,\alpha^2 K(\alpha,m), \tag{20}$$

specific turbidity

$$\frac{\tau}{C} = \frac{3\pi\mu_0}{2d\lambda}\,\frac{m_1 - 1}{m-1}\,\frac{K(\alpha,m)}{\alpha}, \tag{21}$$

where m_1 is the relative refractive index of the pure particle substance, d is its density, and

$$\frac{\tau}{S} = \frac{1}{4} K(\alpha,m), \tag{22}$$

which are expressed by the scattering efficiency factor $K(\alpha,m)$. Note that the specific turbidity τ/ϕ [where $\phi = C(m_1 - 1)/(m-1)d$ is the particle volume concentration] can be used for the determination of particle size[57–59] (or/and refractive index)[23,60] if ϕ (or C[60]) is evaluated from an independent experiment.

2.3 PRINCIPLES OF REGULARIZATION AND THEIR APPLICATIONS

The main regularizing restriction imposed on the function $f(r)$ in this case is, in fact, a requirement of the existence of integrals of the products of $f(r)$ by the corresponding kernels in equations of type (1). It gives one grounds to replace the efficiency factor $K(\alpha,m)$ in relationships (19)–(22) with its analogue K for polydisperse systems calculated by formulae (1), (14), and (15). The next regularization step could be to use the results of calculations of the characteristic light scattering function for model distributions $f(r)$ with specified parameters. Some examples of such calculations are shown in Figure 1 and are also given in Refs. 1, 51, and 61–63. Then, Eqs. (18) and (19) could be used for the determination of a certain mean particle size, which might be used for the following determination of N, C, and S by formulae (20)–(22) when the type of size distribution and the degree of system polydispersity *are known*. For ill-defined systems, however, this approach seems to be ineffective because it is practically impossible to evaluate in advance the parameters of their particle size distribution. The second important aspect of the problem of the method's applicability to real objects is taking into account the effects of particle nonsphericity. For well-defined systems, in principle, one can use the results of calculations of the characteristic functions of light scattering made for particle models of various shapes.[1,2,4–8,36,37,64] Unfortunately, this is left out in reality in the case of ill-defined systems.

It seems to be more advisable for investigations of structurally complex systems to choose universal enough *approximation* expressions for the light scattering characteristic functions and to evaluate their accuracy using one or other models for real systems. The aim of this approach is to exclude, in the general case, the problem of rigorously taking into consideration the parameters of system polydispersity and particle shape, and replacing it by evaluations of limits of these parameters whereby reasonable accuracy of the corresponding approximations is attained. Clearly, approximation potentialities of the "base" model for *monodisperse* systems with *spherical* particles were studied first.[3,29,36,37,39,54] This analysis was made by means of computer simulations, solving the direct problems as analogues of directly measured parameters for model polydisperse systems with spherical particles and systems with randomly oriented prolate and flattened out spheroids, and using the results. Introducing these results in algorithms of solving the inverse problems in which calibration dependences of the characteristic functions of light scattering calculated in the above approximation were used, one can obtain *approximate* values of the system's parameters. Their comparison with the exact data taken into consideration in advance allows one to establish a permissible degree of the system polydispersity or the particle nonsphericity wherein a specified accuracy of the determination of the parameters is ensured. On the other hand, the measure of accuracy fixed (*systematic error*) is of interest as an indication of the closeness of the results of regularized solutions of the inverse problems to those based on a presumable exact solution of the initial integral equation of type (1). In conclusion of the analysis of the effectiveness of the regularization schemes, approximation of light scattering functions was used to evaluate the coefficients of amplification of errors[37] characterizing the general *stability* of the methods of solving the inverse problems.

One of the main problems of the method applicability to real polydisperse suspensions are types of averaging of the particle size. Every type has to satisfy the requirement for each of the light scattering functions used to be the least sensitive to the degree of the system polydispersity.[3,23] Proceeding from this principle and the definitions of the light scattering functions, the following types of averaging were suggested:[3,23,39,54,65]

$$r_\gamma=\left[\int_0^\infty r^{\bar{n}+2}f(r)dr\right]^{1/(\bar{n}+2)} \quad \text{for } \tau/N, \tag{23}$$

$$r_\lambda=\left[\int_0^\infty r^{\bar{n}+2}f(r)dr \Big/ \int_0^\infty r^3 f(r)dr\right]^{1/(\bar{n}-1)}$$
$$\text{for } \tau/C \text{ and } \bar{n}, \tag{24}$$

$$r_\delta=\left[\int_0^\infty r^{\bar{n}+2}f(r)dr \Big/ \int_0^\infty r^2 f(r)dr\right]^{1/\bar{n}}$$
$$\text{for } \tau/S, \tag{25}$$

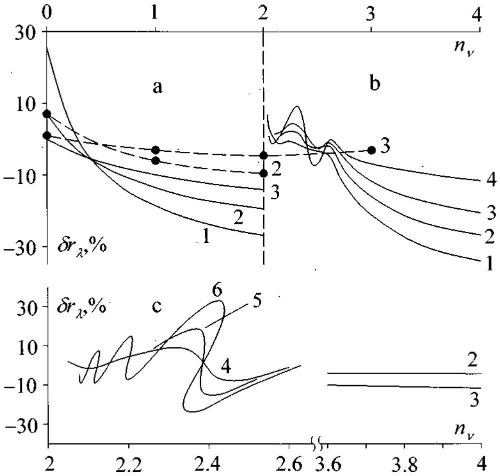

Fig. 3 Dependences of δr_λ on n_ν (Ref. 3) in AD (a) and RGD (b), (c) approximations and for particles with $m=1.33$ (dashed lines) at $\nu=0$ (1), 2 (2), 5 (3), 15 (4), 50 (5), and $\nu\to\infty$ (6).

$$r_\chi=\left[\int_0^\infty r^{\bar{n}+4}f(r)dr \Big/ \int_0^\infty r^{\bar{n}+2}f(r)dr\right]^{1/2} \quad \text{for } \bar{n}, \tag{26}$$

where $\bar{n}$ denotes a certain value of the wavelength exponent for a real or model polydisperse system. The geometrical meaning of these mean radii is established by comparing their expressions and values with standard types of the particle size averaging.[3] It was shown in Ref. 54 that there exist wide areas of mean sizes of Eqs. (23)–(26), where the light scattering functions under consideration are practically invariant with respect to the degree of the system polydispersity described in terms of distribution, Eq. (16). Hence, the turbidity of such suspensions are defined by couples of the corresponding integral parameters (for example, mean size and concentration of particles) practically *independently* of the degree of their polydispersity.

Figure 3 shows some of the results of above mentioned computer simulations characterizing the influence of polydispersity on the results of solutions for the inverse problems where δr_λ is the systematic error of the determination of r_λ. It was established[3] that the algorithms of determination of the mean size and mass–volume concentration had practically no limitations on the degree of the system polydispersity within wide intervals of particle size. At the same time, reliable determination of the particle number concentration imposes restrictions on the degree of polydispersity. Similar estimations were obtained for nonspherical particles.[29,36,37] Some of their examples are shown in Figure 4, where δr and δC are the systematic errors of the determination of r (radius of the sphere of the equivalent volume) and C resulted from the effect of particle nonsphericity. They pointed to the applicability of the method in the case of systems of moderately anisometric randomly oriented par-

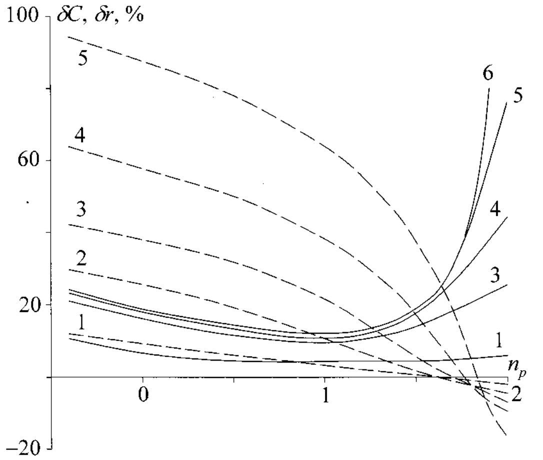

Fig. 4 Dependences of δC and δr (dashed lines) on n_p (Ref. 36) for prolate spheroids in AD approximation at $p=2$ (1), 3 (2), 6 (4), 10 (5), and 15 (6).

ticles (prolate and flattened out) whose axial ratio p is within the interval $1/3<p<3$. The approximation expressions for the smoothed integral light scattering functions, Eqs. (19)–(22), have been worked out on this basis. They permit the algorithms of the inverse problem's solutions to be automated by means of personal computers or even program-controlled pocket microcalculators.[66]

Figure 5 shows the results of utilization of the corresponding software for the determination of the suspension's parameters in a wide interval of n values for various protein disperse systems used as an example. These plots represent the general behavior pattern of reciprocals of the characteristic light scattering functions (being, in fact, similar for various m) depending, to the point, on the particle size: the specific turbidity (for C), the optical cross section (for N), and the scattering efficiency factor (for S). It is evident that while analyzing systems of this (or any other) type in the general case, values of N, C and S determined by such calibrations have to be multiplied by τ.

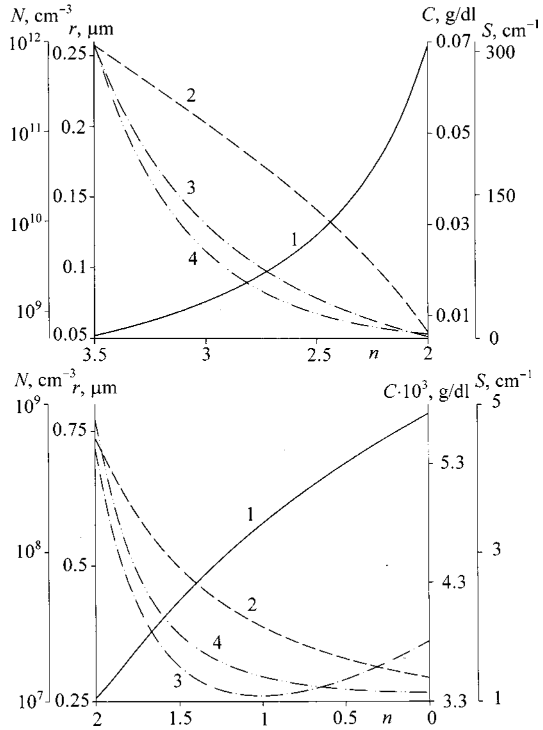

Fig. 5 Calibration plots for the determination of r (1), N (2), C (3), and S (4) by n at $\tau=1$ cm^{-1} ($\lambda_0=0.49$ μm) for protein disperse systems ($d_0=1.3$ g/cm^3, $\mu_0=1.334$, $m=m_1=1.15$).

We return to the problem of taking into consideration the effects of dispersion of the optical constants of particle and medium substances. Making some substantiated assumptions,[52] it is possible to derive the following equations for determination of the particle size or refractive index:[52,65]

$$n(\alpha,m)=n_d+\Delta n, \tag{27}$$

$$\Delta n=\kappa_0 n_d+P\frac{m_1}{m_1-1}(\kappa_1-\kappa_0), \tag{28}$$

$$P=2(n_d\geqslant 2)=n_d(n_d\leqslant 2), \tag{29}$$

$$\kappa_i=d\ln\mu_i\,/\,d\ln\lambda\quad(i=0,1), \tag{30}$$

where $n(\alpha,m)$ is defined by formula (19) and was used, for instance, in Figures 3–5, n_d denotes the value of the wavelength exponent obtained for a real disperse system by relationship (18), κ_i are the experimentally determined features of the optical dispersion of the particle (κ_1) and medium (κ_0) matter. Thus, the correction for the optical dispersion Δn for swelling binary particles, to which the Gladston–Dale law can be applied, does not depend on the degree of particle swelling.

For dispersion media, which are usually practically pure liquids, κ_0 can be found using numerous and detailed results published on spectral measurements of the refractive indices. Unfortunately, for various macromolecular compounds there are no such detailed experimental data. However, it appeared to be possible to use an ease-of-access standard parameter—the reciprocal value of the matter relative dispersion (Abbe number) $\delta=(\mu_{\rm D}-1)/(\mu_{\rm F}-\mu_{\rm C})$, and to obtain the following expression:[52,65]

$$\kappa_1(\lambda_0)=(\mu_{\rm D}-1)(\lambda_{\rm F}^2+\lambda_{\rm C}^2)\,/\,\mu_{\rm D}\delta(\lambda_{\rm F}^2-\lambda_{\rm C}^2), \tag{31}$$

where $\lambda_0=[(\lambda_F^{-2}+\lambda_C^{-2})/2]^{-1/2}$, and the indices C, D, F denote sodium standard spectral lines. The values of δ for more than 100 liquids and polymers, including proteins and carbohydrates, have been published.[67] Using these values, we have constructed the following correlation dependence:[52]

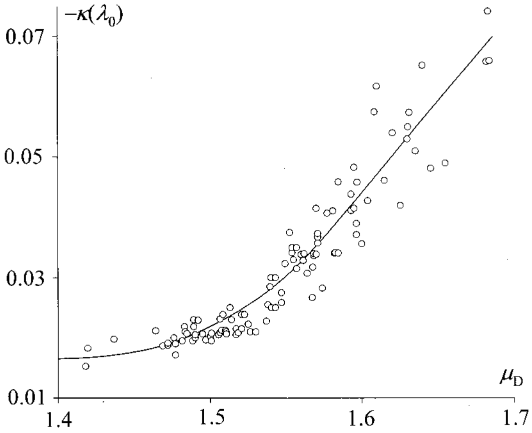

Fig. 6 Dependence of values of $\kappa(\lambda_0)$ on μ_D obtained by formula (31) with the use of experimental data of Ref. 67 (dots) and the correlation curve plotted by formula (32).

$$\kappa_1(\lambda_0)=\sum_{i=0}^{2} B_i(\mu_D-1.4)^i, \qquad (32)$$

where $B_0=0.016\,75$, $B_1=-0.026\,858$, and $B_2=0.780\,829$. This dependence approximates the experimental data with the maximum error of about 20% (Figure 6). Thus, for taking into account the refractive index dispersion it is sufficient to know merely the substance's refractive index for sodium yellow line. Figure 7 presents the dependences of the wavelength exponent on the particle diameter for polystyrene latex suspensions plotted with or without taking into account the dispersion. They were compared with results based on an independent determination of particle size by electron microscopy (dots).[52] It is noteworthy that the dispersion effect most essential for relatively fine suspensions leads to the wavelength exponent values exceeding the limiting theoretical value $n=4$ for Rayleigh particles. The technique developed for particle size determination seems to be applicable for the metrological certification of latex systems. In terms of accuracy, this technique is on par with the electron microscopy method (Table 1), and at the same time, is much less labor-intensive. In Table 1, σ is a mean-square error, d_1 was determined by the electron microscopy method, and d_2 and d_3 were determined by the spectroturbidimetric method, with d_2 and without d_3 taking into account the dispersion $\Delta d=(d_2-d_1)/d_1$.

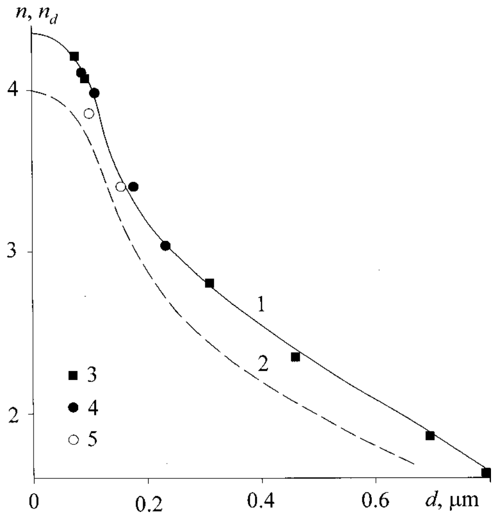

Fig. 7 Dependences of n_d (1) and n (2) on particle diameter d calculated for polystyrene latex suspensions as compared to the experimental data of Table 1 (3), Ref. 68 (4), and Ref. 69 (5).

The general understanding of the spectroturbidimetric method's stability is given by the results of calculations of the coefficients of amplification of errors for the particle size and concentration determination.[37] Figure 8 shows a function k depending on the wavelength exponent, which describes up to a linear factor the coefficients of amplification of errors at different values of m. Within the accuracy of turbidity measurements of about 5%, the maxima of errors in most cases do not exceed admissible values: of the order of 10% for r and C, and 20%–30% for S and N. However, the errors may reach higher values for almost Rayleigh particles ($n\approx4$) whose radius does not exceed approximately 0.05 μm, and for optically soft particles ($m<1.1$) with n values of near 2 as well. The latter

Table 1 Comparison of average diameters d_1-d_3 of the polystyrene latex particles determined by the spectroturbidimetric and the electron microscopy methods (Ref. 52).

Sample	$n_d\pm\sigma$	n	d_1 (nm)	d_2 (nm)	d_3 (nm)	Δd (%)
L1	$4.210\pm9\times10^{-3}$	3.85	76	74	—	−2.6
L2	$4.071\pm2\times10^{-3}$	3.71	91	99	—	8.8
L3	$2.808\pm5\times10^{-4}$	2.46	312	307	210	−1.6
L4	$2.355\pm1\times10^{-4}$	2.02	460	474	340	3
L5	$1.862\pm7\times10^{-4}$	1.55	690	724	564	4.9
L6	$1.621\pm5\times10^{-4}$	1.375	780	817	688	4.7

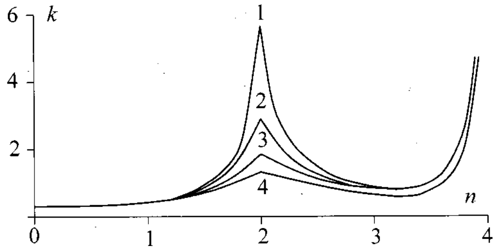

Fig. 8 Dependence of k on n (Ref. 37) at $m=1.05$ (1), 1.1 (2), 1.15 (3), and 1.2 (4).

have radii within the range of approximately 0.2–0.3 μm. Within the bounds of these relatively unfavorable intervals there fall, for example, some bacteria and viruses, certain types of blood cells, some bacterial spores, ribosomes, certain types of liposomes, and membrane vesicles. These cases require either increasing the accuracy of photometric measurements or additional optimization of solutions for the inverse problems.

There are at least two practically important problems whose solutions admit effective optimization, namely, the study of particle agglutination[39,54,55,70] and the evaluation of changes in particle volume induced by particle swelling or shrinkage.[39,54] The agglutination test is one of the most popular diagnostic tools in immunological studies, and volume changes of cells and subcellular particles may provide information on, say, the resistance of membrane structures, some peculiarities of membrane transport, etc. At first sight, the second of the two above problems may seem unsolvable in principle, due to the extreme uncertainty of the system, namely, simultaneous changes in the particle size and refractive index. Nevertheless, it appeared possible to obtain stable solutions by restricting the problem to a reliable evaluation of *relative* changes of particle characteristic parameters and imposing consistent additional prior conditions on the system being studied. References 39, 54, and 70 contain detailed descriptions of this approach. Therefore, we restrict ourselves here to a short discussion of its main results.

When studying cell agglutination, it is assumed that there exists an inversely proportional dependence between the number of agglutinating particles per unit of suspension volume and the mean effective volume of particles (their aggregates). Hence, the following parameter is introduced into consideration: $i=N_1/N_2=r_2^3/r_1^3$ and is given by the meaning of the mean effective number of particles involved in one aggregate. Hereinafter, indices 1 and 2 denote values of the parameters before and after agglutination of particles or changes in their volume. When analyzing volume changes, the inversely proportional dependence between the fractional part of the particle relative refractive index and particle volume is assumed to be the consequent Gladstone–Dale rule: $(\mu-\mu_0)/\mu_0=m-1 \sim 1/r^3$. In both cases, particles are modeled as identical homogeneous spheres. Denoting the relative change in particle volume during swelling or shrinkage as δV with the assumptions made, it is possible to derive the following simple expressions:[39,54,70]

$$i=(D_2/D_1)^{3/(n_0-1)} \quad (n_0\neq 1), \tag{33}$$

$$i=[(2-n_2)/(2-n_1)]^{3/2} \quad (n<2), \tag{34}$$

$$\delta V=[(2-n_1)/(2-n_2)]^{3/4}-1 \quad (n<2), \tag{35}$$

$$\delta V=(D_2/D_1)^{3/(n_0-4)}-1 \quad (n_0\geqslant 2, n_0\neq 4), \tag{36}$$

$$\delta V=(D_2/D_1)^{3/[2(1-n_0)]}-1 \quad (n_0\leqslant 2, n_0\neq 1), \tag{37}$$

where $n_0=(n_1+n_2)/2$. Among the mutually supplementing formulas, Eqs. (33)–(37), one can choose those that are stable at n values of near 2 and even near 4 for the case of agglutination. When using them, there is no necessity for special calibrations and computer processing, taking into account the relative refractive index and other initial particle characteristics (this seems to be very important for structurally complex systems).

We have restricted the consideration to the commonly used agglutination processes, when the number of initial particles in an aggregate does not exceed about 10–20 which is far, e.g., from the fractal regime.[71,72] Detailed calculations were made by Khlebtsov[73,74] for some models for fractal aggregation. In particular, it was shown that measurements of the wavelength exponent n could be used to evaluate the fine structural features of fractal clusters.[73] In addition, these calculations resulted in relationships[74] coincident with formula (33), when the value of fractal dimension F was equal to 3. The use of this F value in the agglutination cases considered was substantiated in Ref. 54. This probably may serve as additional evidence for a conclusion given in Refs. 75 and 76 that the useful range of the theory of homogeneous particles is wider than is usually presumed.[76]

3 PRINCIPLES OF SPECTROTURBIDIMETRY OF ORIENTATION-ORDERED DISPERSE SYSTEMS

As the data presented above suggest, in the general case of *randomly* oriented nonspherical particles, a spectroturbidimetric experiment may result in the following set of suspension parameters: r, C, N and S, with μ and p being known in advance; or μ, C (or r), N and S, with r (or C) and p being known in advance. The need for a prior assessment of the particle refractive index, size (or concentration) and axial ratio by independent techniques (e.g., microscopic, photometric, immersion–photometric, etc.)

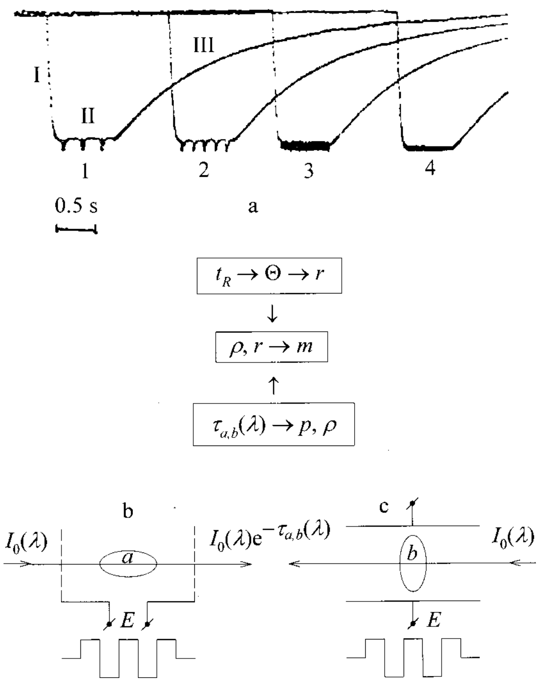

Fig. 9 Oscillograms of electro-optical signals (a) for *E. coli* cell suspension (Ref. 77) at pulse frequencies of 2.5 (1), 7 (2), 14 (3), 25 (4) Hz and a scheme (b), (c) of p, ρ and m determination.

essentially complicates the spectroturbidimetric method and lays constraints on its accuracy.

It was shown in Refs. 77 and 78 that the effect of particle orientation in combination with the effect of orientation relaxation and the results of measurements at random particle orientation may serve as a source of additional information about the system's structure. Using algebraic terminology, this approach means increasing the number of independent equations used for the determination of structural parameters of disperse systems. It allows one to increase the number of these parameters (including axial ratio, size, and refractive index of particles) being determined in a *single* combined experiment. Principles of spectroturbidimetry of the orientation-ordered disperse systems are considered here with a combination of the spectroturbidimetry and an electro-optical technique used as an example. Note that the electro-optical properties of suspensions serve, in particular, for estimations of the most important electrophysical parameters of suspended particles and find wide application in biophysical, biochemical, and physicochemical investigations of various suspensions.[16–21,53,56,79]

Figure 9(a) presents oscillograms[77] reflecting temporal changes in the transparency of an *Escherichia coli* cell suspension under the action of bursts of rectangular pulses of an orienting electric field in unpolarized light ($\lambda = 810\,\mathrm{nm}$), directed perpendicularly to the orienting field with an amplitude E of 100 V/cm [Fig. 9(c)]. An electro-optical signal consists of three parts, corresponding to the relatively fast clarification of the suspension (I), stationary part, corresponding to a given degree of particle orientation (II), and relaxation part, corresponding to the transition of the suspension to the state of random particle orientation with a time of relaxation t_R (III) (820 ms in these experiments). At relatively low pulse frequencies [curves 1–3 in Figure 9(a)], signal modulations are observed in the region of stationary values of transmission which can be explained by cell low-amplitude oscillations about the direction of the particles' primary orientation.[80] The turbidity spectra $\tau_{a,b}$ corresponding to the stationary [Figure 9(a)] parts and measured at the mutually perpendicular directions of the orienting field [Figures 9(b), (c)] can be used to determine the axial ratio $p = a/b$ and phase shift parameter ρ by appropriate formulae and calibrations.[77]

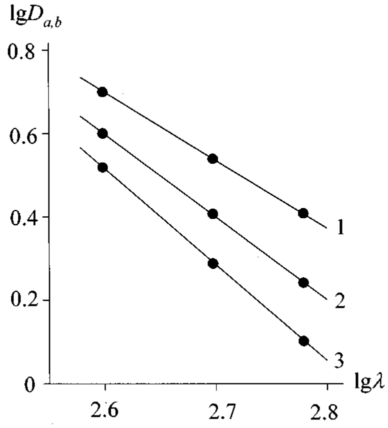

Fig. 10 Plots of log $D_{a,b}$ vs log λ for *E. coli* suspensions (Ref. 77) with ordered (1, 3) and random (2) cell orientation.

Figure 10 presents double logarithmic plots of the turbidity spectra of *E. coli* suspensions for three different states of particle orientation. Changing the slope of these plots from $n_b = 2.32$ (plot 3) to $n_a = 1.67$ (plot 1) reflects the increase in the central ray phase shift with the change in particle orientation. The value $n = 2$ (plot 2) was obtained in the case of random particle orientation. It is worth noting that, if prolate optically soft particles are large enough and their ρ values are small ($n \approx 2$), the axial ratio p is just equal to τ_a / τ_b, while in the more general case (unlimited ρ values) $p = (\tau_a / \tau_b)^{1/(n-1)}$. For instance, many bacterial suspensions satisfy these conditions. As a source of additional particle size information, the coefficient of rotary diffusion Θ ($0.2\,\mathrm{s}^{-1}$ in this case) determined by the relaxation curves is used. When combining the results of the independent determinations of the particle size r and the phase shift parameter ρ, one can determine the particle relative refractive index m[77] (see Figure 9). Finally, the orientation turbidimetric effect,[20] i.e., increments of turbidity $\Delta\tau_{a,b}$ corresponding to the stationary parts of the electro-optical signals, nor-

Table 2 Results of determination of *E. coli* cell parameters by the spectroturbidimetric (1) and independent (2) methods.

Parameter	ρ	r (μm)	p	m	μ
1	0.95	0.6	2.45	1.046	1.39
2	—	0.64^a	2.5^a	1.035^b	1.38^b
Parameter	$N\times 10^{-6}$ (ml^{-1})	C (μg/ml)	S (cm^2/ml)	$\Delta\gamma\cdot 10^{29}$ (F m^2)	—
1	15	34	6.9	4	—

[a] Light microscopy.

[b] Immersion photometry (Refs. 50 and 82).

malized to the values of τ obtained at random particle orientation, is utilized. Its dependence on the strength of the orienting electric field E obtained experimentally and calculated theoretically (with due account of the particle parameters determined) can be used for the evaluation of particle surface polarizability $\Delta\gamma$. The latter is defined as the $\Delta\gamma$ value which gives the best accord between theory and experiment.[20,77] Table 2 gives an example of the use of the method considered[77] corresponding to the data of Figures 9 and 10.

Thus, combining the stationary and relaxation *spectral* characteristics of the electro-optical effect in the cases of random and ordered particle orientation, it is possible, in principle, to obtain *in situ* the following set of suspension's characteristics: (1) mean particle size; (2) refractive index; (3) axial ratio; (4) the number of particles per unit volume of the suspension; (5) particle mass–volume concentration; (6) the total surface area of suspended particles per unit volume of the suspension; and (7) the effective value of particle electrical surface polarizability. Notice that the general approach and the data described in this section provided the basis for further development of effective algorithms of solving the inverse problems in a wide range of particle sizes, axial ratios, and refractive indices. Specifically, the basic problems of interpretation of relaxation measurements[21,81] were considered, and iteration procedures making (when necessary) more accurate results of the determination of particle parameters were developed[78] by the use of the T-matrix method.[6,26,27] A prototype *electro-optical spectroturbidimeter*, which can be used in particular to create the optimal conditions for experimental realization of the above potentials, was designed and made on the basis of collaboration between IBPPM RAS, the Samara Medical Institute (Samara), and the State Research Institute of Applied Microbiology (Obolensk).

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