copolymer of PI-VP, ΔS^* is characterized by large negative values of −58.0 eu. For MI-VP polymer, ΔV^* values are −20 cm^3/mol for NBBA and NPeBA, irrespective of the alkyl chain length of the substrates, and −19 cm^3/mol for PNPA as shown in Table II. If the rate-limiting step were the acylation of the polymer catalyst, the observed activation parameters would correspond to the acylation process in eq 13 since $k_{\text{acyl}} \ll k_{\text{deacyl}}$.

Kunitake and Shinkai[14] concluded that the large negative ΔS^* of −58 eu was caused primarily by the rupture of the hydrophobic interaction in the transition state for the PI-VP system. However, it is expected from the negative ΔV^* that the intracomplex process of MI-VP is predominantly caused by the increasing polarity in the transition state. The ΔV^* of −16 cm^3/mol accompanying the hydrolysis of PNPA catalyzed by Im free from the hydrophobic interaction is due to the polarity increase in the transition state and only the difference of −4 cm^3/mol ($= \Delta V^*_{\text{MI-VP}} - \Delta V^*_{\text{Im}}$) would be expected to be caused by the rupture of the hydrophobic interaction in the transition state. As this amount is between a half and one third of the contribution to the hydrophobic interaction of the substrate binding, the hydrophobic interaction still partially exists in the transition state of the MI-VP system. It is concluded that these negative values of ΔV^* may be attributed mainly to polarity increase and partly to the breaking of the hydrophobic interaction.

The entropy of the activation accompanying the deacylation process from the study of the hydrolysis of acetylimidazole is −30.2 eu.[21] This result strongly favors a rate-limiting bimolecular attack of water on the acetylimidazolium cation rather than a monomolecular formation of acetylium cation. The activation volume of −16.0 cm^3/mol for the Im-catalyzed reaction also seems to correspond to the deacylation process, because of the same rate both of acylation and deacylation steps and the large negative entropy of activation. If the rate-limiting step were the deacylation process in the MI-VP systems, the activation volume of −20 cm^3/mol, irrespective of the alkyl chain length of the substrates, would be explained to be the bimolecular attack of water on the alkylimidazolium. On the other hand, the ΔV^* accompanying the deacylation process of acyl-α-chymotripsin[22] depends on the kinds of phenyl esters of aliphatic acid, which shows −6 cm^3/mol for acetic, −3 cm^3/mol for dimethylacetic, −2 cm^3/mol for trimethylacetic acids, respectively. Catalysis by α-chymotripsin belongs to the general acid–base catalysis with Im (His-57) inducing a proton transfer from the hydroxy group (Ser-195) to the carboxylic acid (Asp-102). Therefore, the large difference between the ΔV^* values of the hydrolysis of phenyl esters catalyzed by synthetic polymer containing Im and by α-chymotripsin is due to the difference of the reaction mechanism. Further study is required to probe the meaning of the difference of ΔV^*.

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References and Notes

(1) Presented in part at the 4th International Conference on High Pressure, Kyoto, Japan, Nov. 25–29, 1974.
(2) K. J. Laidler and A. J. Beardell, *Arch. Biochim. Biophys.*, **55**, 138 (1955).
(3) B. Andersen and P. E. Broe, *Acta Chem. Scand.*, **26**, 3691 (1972).
(4) K. C. Mohankumar and L. R. Berger, *Anal. Biochem.*, **49**, 336 (1972).
(5) R. K. Williams and C. Shen, *Arch. Biochem. Biophys.*, **152**, 606 (1972).
(6) W. M. Neville and H. Eyring, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2417 (1972).
(7) T. Kunitake, F. Shimada, and C. Aso, *J. Am. Chem. Soc.*, **91**, 2716 (1969).
(8) K. Suzuki and Y. Taniguchi, *Symp. Soc. Exp. Biol.*, **26**, 103 (1972).
(9) F. J. Kezdy and M. L. Bender, *Biochemistry*, **1**, 1097, (1962).
(10) F. Takahashi and S. Otuka, *Nippon Kagaku Zasshi*, **91**, 815 (1970).
(11) K. Suzuki, Y. Taniguchi, and K. Shimokawa, The 14th Symposium on High Pressure, Japan Chemical Society, Osaka, 1972, p 140.
(12) P. Molyneux and H. P. Frank, *J. Am. Chem. Soc.*, **83**, 3169 (1961).
(13) ΔS_u is the unitary entropy given by the equation of $\Delta S_u = \Delta S + 7.98$.
(14) T. Kunitake and S. Shinkai, *J. Am. Chem. Soc.*, **93**, 4256 (1971).
(15) K. Suzuki, Y. Taniguchi, and Y. Watanabe, *J. Phys. Chem.*, **77**, 1918 (1973).
(16) K. Gekko and Y. Noguchi, *Macromolecules*, **6**, 438 (1973).
(17) M. E. Friedman and H. A. Scheraga, *J. Phys. Chem.*, **69**, 3795 (1965).
(18) D. M. Bouwer, M. J. Vlugt, and E. Havinga, *Proc. K. Ned. Akad. Wet., Ser. B*, **60**, 275 (1957).
(19) M. L. Bender and B. Turnquest, *J. Am. Chem. Soc.*, **79**, 1656 (1957).
(20) T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.*, **79**, 1663 (1957).
(21) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272 (1957).
(22) R. C. Neuman, Jr., D. Owen, and G. D. Lockyer, Jr., *J. Am. Chem. Soc.*, **98**, 2982 (1976).

Photon-Count Autocorrelation Spectroscopy. Data Analysis in the Case of Monoexponential Spectra

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ABSTRACT: A series of monoexponentially decaying single clipped photon autocorrelation spectra of the laser light quasielastically scattered by monodisperse solutions of macromolecules was analyzed in different ways to find out how the relaxation times, which are related to the diffusion coefficient of the macromolecules, can be determined accurately in spite of distortions of the spectra. Following various computer fitting procedures different values for relaxation times and quality parameters were obtained from the individual spectra; the best estimate for the relaxation time was then found from a plot which combined all the results.

Diffusion coefficients of macromolecules can be determined accurately by intensity fluctuation spectroscopy.[1] Laser light scattered by a dilute, monodisperse solution of macromolecules which are small compared to the wavelength of light yields the intensity autocorrelation function

$$G^{(2)}(iT) = A' \exp(-\Gamma iT) + C$$

with A' the amplitude, which depends on the geometry of the experimental setup,[2] i a number varying from 1 to the total number of channels of the correlator, T the sample time, C a

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Table I
Description and Results of Measurements and Data Analysis

Solution no.	1			2	
Temp, °C	30			25	
Scattering angle, deg	90			90	135
No. of spectra	18			18	14
	$\Gamma T \times 10^3$ (145)[a]	χ^2	$A \times 10^3$	$\Gamma T \times 10^3$ (96)	$\Gamma T \times 10^3$ (131)
$g^{(2)}_k(iT) - 1 = A \exp(-\Gamma iT) + \delta$	145 ± 2	47 ± 23	480 ± 4	95.4 ± 1.2	129 ± 2
$\ln [g^{(2)}_k(iT) - 1] = \ln A - \Gamma(iT)$	131 ± 3	71 ± 54	456 ± 10	94.7 ± 1.4	124 ± 2
$\ln [g^{(2)}_k(iT) - 1] = \ln A - \Gamma(iT) + X(iT)^2$	148 ± 6	69 ± 61	488 ± 8	95.3 ± 2.7	128 ± 3
$\ln [g^{(2)}_k(iT) - 1] = \ln A - \Gamma(iT) + X(iT)^2 + Y(iT)^3$	141 ± 8	33 ± 22	481 ± 8	95.9 ± 3.0	131 ± 6

[a] The values of $\Gamma T = 2DK^2T$ calculated from the Stokes–Einstein relation are indicated in parentheses. .

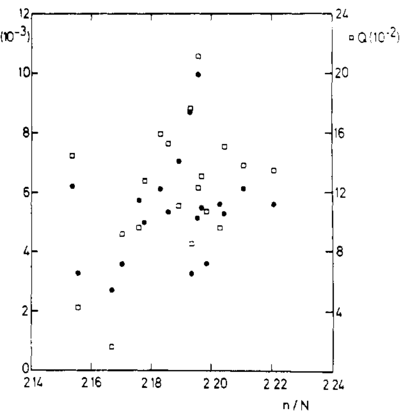

Figure 1. The mean number of counts per sample time n/N as a function of δ (●) and Q (□), which indicate deviations from monoexponential decay, for 18 autocorrelation spectra of the light scattered at 90° by solution 1 of polystyrene latex spheres.

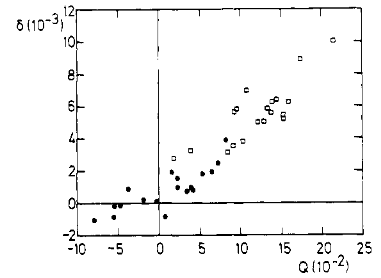

Figure 2. Correlation between the quality parameters δ and Q of autocorrelation spectra of the light scattered at 90° by two different solutions of polystyrene latex spheres: 1 (●) and 2 (□) (cf. Table I).

constant background, and $\Gamma = 2DK^2$, where D is the diffusion coefficient of the macromolecules and K the modulus of the scattering vector. In particular, the efficient single clipped photon-count autocorrelation spectroscopy yields the normalized correlation function

$$g^{(2)}_k(iT) = A \exp(-\Gamma iT) + 1$$

where k indicates the clipping level.[3]

However, quasielastic light scattering in general is complicated by the presence of "dust" in the scattering volume (see, e.g., ref 4). Therefore the spectra $g^{(2)}_k(iT)$ have been fitted according to

$$\ln [g^{(2)}_k(iT) - 1] = \ln A - \Gamma(iT) + X(iT)^2$$

from which the quality parameter $Q = 4X/\Gamma^2$ could be derived; spectra for which $|Q|$ exceeded a certain value (e.g., $|Q| \geqslant 0.02$) were then rejected.[5] We found that all the data obtained from linear fits can be taken into account by combining them in a plot of D vs. Q^6 or equivalently of ΓT vs. Q, which gives then the best estimate for ΓT at $Q = 0$ (as illustrated further in Figure 3). Analogously, it was reported in this journal[7] that the diffusion coefficient of polystyrenes could be obtained accurately by fitting spectra according to

$$g^{(2)}_k(iT) - 1 = A \exp(-\Gamma iT) + \delta$$

Γ was then almost independent of the small "misnormalization" δ.

Here we compare the results of the application of different methods for the analysis of the monoexponentially decaying correlation spectra $g^{(2)}_k(iT)$ of the light scattered by dilute solutions of small monodisperse polystyrene latex spheres, which were contaminated by dust. It is concluded that both Q and δ can serve as quality parameters in Q, ΓT and/or δ, ΓT plots from which an accurate value of Γ can be derived in spite of the distortions of the spectra.

Experimental Section

Laser light was scattered by aqueous, dilute solutions of monodisperse polystyrene latex spheres (Dow Chemical Co.) with a diameter of 91 nm to obtain monoexponentially decaying autocorrelation spectra. The cylindrical, glass Malvern scattering cells were placed in a Malvern index matching glass bath which contained dust-free water and was thermostated at 30.0 or 25.0 ±0.1 °C with a Malvern temperature controller. Three series (see the upper part of Table I) of single clipped photon-count autocorrelation spectra[3] were taken with a Malvern photon correlation spectrometer which included an intensity stabilized coherent radiation argon ion laser, operating at 488 nm, and a ITT FW130 photomultiplier. Each measurement yielded 24 unnormalized spectrum points $G^{(2)}_k(iT)$ (with $i = 1, \ldots, 24$), the total number of counts n, clipped counts n_k, and sample times N. All data were recorded on paper tape and analyzed using a PDP 11/45 computer. First $g^{(2)}_k(iT)$ was fitted as summarized in the first column of Table I to obtain ΓT. The first direct fit to an exponential curve plus a background $\delta \approx 0$ was based on an iterative procedure.[8] Also linear fits were performed of $\ln [g^{(2)}_k(iT) - 1]$ to polynomial curves of different order.[9]

Purified PP7 virus in 15 mM Tris/HCl buffer (pH 7.2), 150 mM NaCl, and 1 mM $MgCl_2$ was a gift of Dr. M. Van Montagu. The particles were further subjected to a zonal centrifugation through a linear 10 to 30% sucrose density gradient in a Spinco SW27 rotor in this laboratory. The top fractions were collected and dialyzed exhaustively. The solutions were diluted with buffer which was made dust free by centrifugation. Prior to light scattering the cell was put in a special adapter and centrifuged at 6000 rpm for 1 h in a Spinco JA20 fixed-angle rotor. The temperature during the measurements was 25.0 °C.

Results and Discussion

Dust particles in the scattering volume contribute to the number of photon counts in every measurement. However, Figure 1 shows that a high n/N does not always reflect the distortion of a spectrum which is indicated by nonzero δ and Q. So n/N should not be used as a quality parameter. Figure 2 shows that δ and Q correlated well. Thus both of these quality parameters can be used to decide whether a measurement should be rejected or not, but they can better be

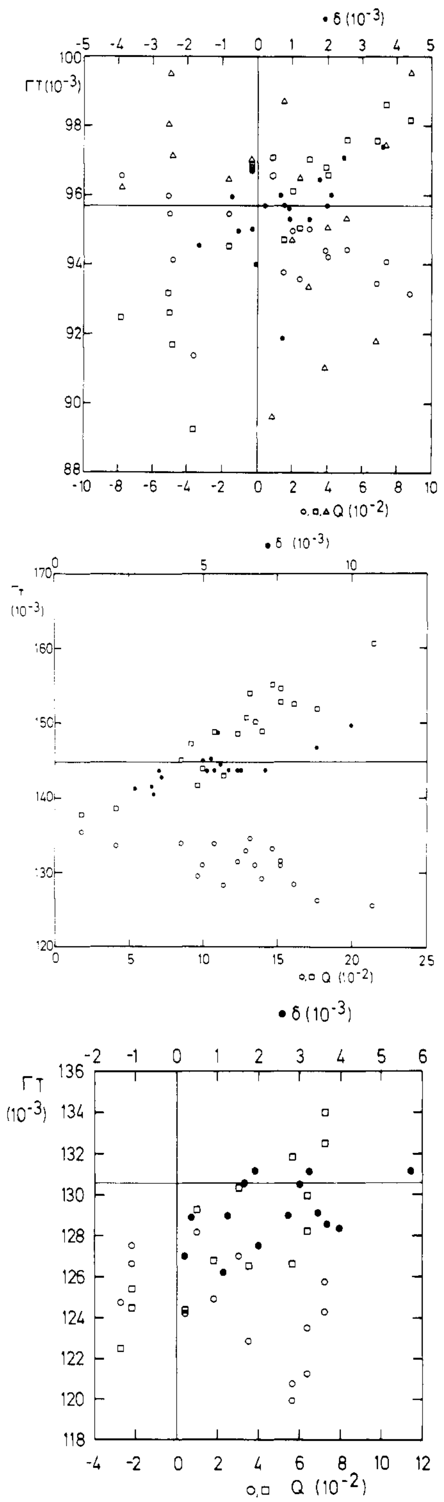

Figure 3. Decay rates ΓT of three series of autocorrelation spectra $g^{(2)}{}_k$ of the light scattered by polystyrene latex spheres as a function of the corresponding quality parameters δ and Q. Values of ΓT obtained after fitting of $g^{(2)}{}_k - 1$ to a monoexponential curve plus background δ were plotted as a function of δ (●) and those obtained after fitting $\ln [g^{(2)}{}_k - 1]$ to a straight line (○), a quadratic (□), or a cubic curve (▵) were plotted as a function of Q: (a) solution 1, scattering angle 90°; (b) solution 2, scattering angle 90°; (c) solution 2, scattering angle 135°. The horizontal lines indicate the respective values for ΓT calculated from the Stokes–Einstein equation.

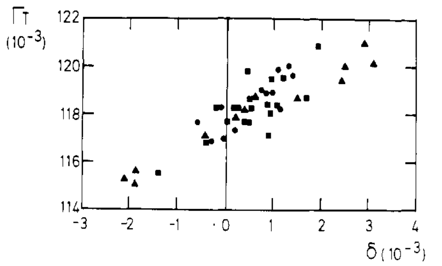

Figure 4. Decay rates ΓT of autocorrelation spectra of the light scattered at 90° by solutions of PP7 virus as a function of their "misnormalization" δ. Concentrations were 0.44 (●), 0.23 (■), and 0.15 (▲) mg/mL.

used so that all the spectra are taken into account by plotting the data as shown in Figure 3; the best value for ΓT is then found from an interpolation or extrapolation to $\delta = Q = 0$. As the spread of the values for ΓT obtained from the fits to cubic curves was too large, they were only plotted in Figure 3a and fits to polynomial curves of higher order were not performed. It is seen that values of ΓT from the fits of $\ln [g^{(2)}{}_k(iT) - 1]$ to straight lines tend to decrease with Q, while those from the fits to quadratic curves tend to increase with Q; this confirms our earlier observations.[6] The values of ΓT obtained from the direct fits of every spectrum to an exponential curve plus background δ show only a relatively small dependence of δ; this is in agreement with the findings of Lacharojana and Caroline.[7] When the possibility to make plots such as in Figure 3 is not exploited and mean values are simply calculated from all the data, one obtains the results summarized in Table I. Inclusion of a small background δ in $[g^{(2)}{}_k(iT) - 1]$ (or alternatively a small deviation $X(iT)^2$ of $\ln [g^{(2)}{}_k(iT) - 1]$ from a straight line) clearly yields better results than fitting $[g^{(2)}{}_k(iT) - 1]$ to a pure exponential curve. The autocorrelation function need in fact not even be normalized for the fitting to an exponential curve plus background; so n, n_k, and N need not be determined.

Following the data analysis procedure for monoexponential autocorrelation spectra with plots as shown in Figure 3, the available information is used more efficiently than when more simple methods are applied. Therefore it has been used in this laboratory in the determination of the diffusion coefficient of bacteriophage MS2,[10] Artemia salina ribosomes,[11] and small, spherical RNA viruses; Figure 4 is a typical plot, made for the determination of the diffusion coefficient and its eventual concentration dependence of PP7 virus in 15 mM Tris/HCl buffer (pH 7.2), 150 mM NaCl, and 1 mM $MgCl_2$.

Our computer program in Fortran IV is available on request.

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References and Notes

(1) H. Z. Cummins and P. N. Pusey in "Photon Correlation Spectroscopy and Velocimetry", Plenum Press, New York, N.Y., 1977.
(2) P. Nieuwenhuysen and J. Clauwaert, *Rev. Sci. Instrum.*, **48**, 699 (1977).
(3) R. Foord, E. Jakeman, C. J. Oliver, E. R. Pike, R. J. Blagrove, E. Wood, and A. R. Peacocke, *Nature (London)*, **227**, 242 (1970).
(4) O. Kramer and J. E. Frederick, *Macromolecules*, **4**, 613 (1971).
(5) P. N. Pusey, D. E. Koppel, D. W. Schaefer, R. D. Camerini-Otero, and S. H. Koenig, *Biochemistry*, **13**, 952 (1974).
(6) P. Nieuwenhuysen and J. Clauwaert in Abstracts, 1976 Prague Meetings on Macromolecules, M 22, and *J. Polym. Sci., Part C*, in press.
(7) S. Lacharojana and D. Caroline, *Macromolecules*, **10**, 365 (1977).
(8) G. R. Deily, *Commun. ACM*, **9**, 85 (1966).
(9) P. R. Bevington, "Data Reduction and Error Analysis for the Physical Sciences", McGraw-Hill, New York, N.Y., 1968.
(10) P. Nieuwenhuysen and J. Clauwaert, *Biopolymers*, accepted for publication.
(11) J. Clauwaert and P. Nieuwenhuysen in Abstracts, 12th FEBS Meeting, Dresden, Abstract No. 3543; P. Nieuwenhuysen and J. Clauwaert, *Biochemistry*, accepted for publication.