Relation between the initial slope of some electro-optic decays and the moments of the distribution of size

H. J. Coles and G. Weill
Centre de Recherches sur les Macromolécules, CNRS and Université L. Pasteur, Strasbourg, France
(Received 27 June 1977)

Several well defined average values of the rotatory diffusion constant D of a polydisperse system can be obtained using the initial slope of the free decay of different electro-optical phenomena and different degrees of orientation. The type of average depends also on the orientation mechanism. The type of average and its relation to the corresponding moments of the size distribution, which have been previously misused, are derived for rod-like and disc-like particles, different mechanisms of orientation, and two types of electro-optical effect: birefringence (or dichroism) and light scattering in an electric field. Re-examination of the agreement claimed by previous authors between averages of D measured by this technique and calculated from a distribution of size derived from electron micrographs illustrate the difficulties of the measurements at saturation of orientation, and the role of an exact knowledge of the orientation mechanism. Possible experiments are discussed. Two parameters analytical distribution functions, well suited for the expression of the differen. moments appearing in the theory, are indicated.

INTRODUCTION

Many methods have been devised to derive the 'spectrum of relaxation times' or some of its first moments from the field free decay following the switch off of the electric field in electro-optical measurements. They range from a simple analysis in terms of a superposition of a small number of exponential decays whose decompositions can be carried out graphically by the 'peeling method'[1] to elaborate numerical methods for inverse Laplace transforms[2,3]. In the case of suspensions of colloid particles or solutions of rigid polymers the method has been widely used to evaluate the polydispersity. The problem of relating the 'distribution of relaxation times' to the 'distribution of sizes' has very often been overlooked and it has not always been realized that it depended not only on the relation between the size and the rotatory diffusion constant D but also on the electro-optical effect under consideration. Following an early paper of Kobayashi[4] Schweitzer and Jennings[5] have carefully discussed this latter point for rod-like particles and shown that several well defined average values of the rotatory diffusion constant can be measured from the initial decay slope when changing the electro-optical effect and/or the degree of orientation. Unfortunately they did not relate these averages to the proper average values of the sizes. The exceptional agreement obtained in a subsequent paper by Foweraker and Jennings[6], when comparing experimental D averages to those calculated from average sizes deduced from electron micrographs, on suspension of rod-like particles, is therefore puzzling. It is the purpose of this paper (i) to restate the theory in a way which makes it applicable to more general cases; (ii) to give the proper relation between the averages of the rotatory diffusion constant and the moments of the distribution of size; (iii) to re-examine and reinterpret the published data.

DECAY PROCESSES IN POLYDISPERSE SYSTEMS

For a discrete distribution of particles, the decay of any optical effect Z after the pulse has terminated can be written:

$$Z(t) = \Sigma_i \Sigma_n Z_{in}(0)\exp(-n(n+1)Dt) \qquad (1)$$

where the first sum is over all particles i, and the second sum arises from the development of the orientation function in terms of Legendre polynomials P_n. In the case of small orientation this sum is limited generally to the term $n = 2$. This remains true at large orientation for electric birefringence or electric dichroism[7] but may no longer be true for light scattering[8,9] or polarization of fluorescence[10] in an electric field. In the following analysis we shall be restricted to the case where:

$$Z(t) = \Sigma_i Z_i(0)\exp(-6D_i t) \qquad (2)$$

For a continuous distribution one can write:

$$Z(t) = \int y(D)e^{-6Dt}\mathrm{d}D \qquad (3)$$

and use the properties of Laplace transforms to obtain $y(D)$ or its first moments. As previously stated the relation of y to the size distribution $n(l)$ is by no means simple as appears from the following development.

SIZE DEPENDENCE OF THE STEADY STATE ELECTRO-OPTICAL EFFECT

Going back to the discrete distribution and following Kobayashi[4] $Z_i(0)$ can be expressed as the product of three size dependent factors:

$$Z_i(0) = c(l_i)g(l_i)\phi(l_i) \qquad (4)$$

where c is the weight concentration in particles of size l_i; g is the 'optical factor' i.e. the difference in principal polarizabilities by unit volume for birefringence, the difference in absorbency along and perpendicular to the symmetry axis for dichroism[11], the product of the molecular weight with the change in the interference factor for light scattering[12,13]; ϕ is the orientation function which depends on both l and the applied electric field.

In many cases, and particularly for rods of small diameter or discs of small thickness, c, g and ϕ can be taken as proportional to some power of l, as recognized for rods by Kobayashi and emphasized by Jennings and coworkers[5,6]. Specific cases are given below for rod-like (l being the length) and disc-like (l being the radius) particles:

(a) $c(l) \propto n(l) \times l$ for rods of uniform diameter;
$c(l) \propto n(l) \times l^2$ for discs of uniform thickness.

(b) $g(l)$ is independent of l for birefringence and dichroism; $g(l)$ is for light scattering in the range of small Kl where equation (2) is fulfilled ($K = (2\pi/\lambda) \sin \theta/2$, with θ the scattering angle and λ the wavelength in the scattering medium), proportional to $M_i l^2$ [17,13] i.e. (M_i is the molecular weight of the ith particle):

$g(l) \propto l^3$ for scattering by rods

$g(l) \propto l^4$ for scattering by discs

(c) $\phi(l)$ is independent of l at very high fields where saturation of orientation is reached. In the Kerr region it depends on the orientation mechanism:

(i) orientation resulting from the anisotropy of the electronic polarizability (as in the orientation by a laser field[14]):

$\phi(l) \propto l$ for rods

$\phi(l) \propto l^2$ for discs

(ii) orientation resulting from an axial permanent moment:

$\phi(l) \propto l^2$ for rods

$\phi(l) \propto l^4$ for discs

(iii) orientation resulting from the anisotropy of an ionic polarizability (polyelectrolytes and colloids):

$\phi(l) \propto l^3$ for rods with very weak counterion–counterion repulsion in the ionic atmosphere[15]

$\phi(l) \propto l^2$ for rods with strong counterion–counterion repulsion in the ionic atmosphere[15,16]

We can therefore take:

$$c(l) = n(l) l^{\alpha} \qquad g(l) \propto l^{\beta} \qquad \phi(l) \propto l^{\gamma} \tag{5}$$

γ corresponds to a in the notation of ref 5, where α, β and γ depend on the shape, the electro-optical effect and the degree of orientation. Introducing equation (5) into equations (4) and (2) one has:

$$\frac{Z(t)}{Z(0)} = \frac{\Sigma l_i^{\alpha+\beta+\gamma} n_i \exp(-6D_i t)}{\Sigma l_i^{\alpha+\beta+\gamma} n_i} = \frac{\int l^{\alpha+\beta+\gamma} n(l) \exp(-6D(l)t) \mathrm{d}l}{\int l^{\alpha+\beta+\gamma} n(l) \mathrm{d}l} \tag{6}$$

MEAN VALUES OF THE ROTATORY DIFFUSION CONSTANT AND ASSOCIATED MEAN SIZES

From equation (6) one calculates the initial slope:

$$\frac{\mathrm{d}}{\mathrm{d}t}\left[\frac{Z(t)}{Z(0)}\right]_{t=0} = \left(\frac{\mathrm{d}\log Z}{\mathrm{d}t}\right)_{t=0} = \frac{-6\int l^{\alpha+\beta+\gamma} D(l) n(l) \mathrm{d}l}{\int l^{\alpha+\beta+\gamma} n(l) \mathrm{d}l} \tag{7}$$

which is therefore a well defined average over the distribution of length. At this point, Schweitzer and Jennings[5] define different average values according to the value of $p = \alpha + \beta + \gamma$. For rods where $M \propto l$:

$$D_N = \int D(l) n(l) \mathrm{d}l / \int n(l) \mathrm{d}l$$
$$D_w = \int D(l) l n(l) \mathrm{d}l / \int l n(l) \mathrm{d}l \tag{8}$$
$$D_Z = \int D(l) l^2 n(l) \mathrm{d}l / \int l^2 n(l) \mathrm{d}l$$

In a following paper[6] the decay of birefringence of solutions of rod-like particles ($\alpha = 1$, $\beta = 0$) was measured in the saturation ($\gamma = 0$) and in the Kerr region (under the assumption of an orientation mechanism such that $\gamma = 1$). These authors therefore obtain a D_w ($p = 1$) and a D_Z ($p = 2$) average which can be compared with some average length:

$$\bar{l}_q = \frac{\int l^q n(l) \mathrm{d}l}{\int l^{q-1} n(l) \mathrm{d}l} = \frac{\langle l_q \rangle}{\langle l_{q-1} \rangle} \tag{9}$$

where $\langle l_q \rangle$ is the q^{th} moment of the distribution in length ($l_1 = l_N, l_2 = l_w, l_3 = l_Z, \ldots; l_0 = l_{N-1}, l_{-1} = l_{N-2}, \ldots$) using Burgers formula[17]:

$$D = \frac{3kT[\ln(l/d) - 0.8]}{\pi \eta_0 l^3} \tag{10}$$

While the authors explicity state that 'an equation such as equation (10) does not necessarily relate a specific $\bar{l}_q$ to the q average of D owing to the presence of these parameters to different powers in the equation', they claim that 'the disparity is not likely to be large compared to experimental error' and obtain an amazingly good agreement when comparing D_w and D_Z with values calculated from equation (10) with l_w and l_Z as obtained from histograms built from electron micrographs.

We will now show that the proper average length which has to be introduced in equation (10) for comparison can be predicted theoretically and that the disparity is likely to be large.

Indeed from equation (10) one sees that D is essentially related to l by $D \propto Al^{-3}$. A similar relation holds for discs of negligible thickness[18]:

$$D = \frac{3kT}{32\eta l^3} \tag{11}$$

Table 1 Average sizes associated with rotatory diffusion constants as measured from the decay of two electro-optical effects

Rod-like particles ($\alpha = 1, \delta = 3$)				Disc-like particles ($\alpha = 2, \delta = 3$)			
Orientation conditions		Birefringence and dichroism $\beta = 0$	Light scattering in electric field $\beta = 3$	Orientation conditions		Birefringence and dichroism $\beta = 0$	Light scattering in electric field $\beta = 4$
Saturation	$\gamma = 0$	$[\bar{l}_{N-2}\bar{l}_{N-1}\bar{l}_N]^{1/3}$	$[\bar{l}_w\bar{l}_Z\bar{l}_{Z+1}]^{1/3}$	Saturation	$\gamma = 0$	$[\bar{l}_{N-1}\bar{l}_N\bar{l}_w]^{1/3}$	$[\bar{l}_{Z+1}\bar{l}_{Z+2}\bar{l}_{Z+3}]^{1/3}$
E^2 region*	$\gamma = 1$	$[\bar{l}_{N-1}\bar{l}_N\bar{l}_w]^{1/3}$	$[\bar{l}_Z\bar{l}_{Z+1}\bar{l}_{Z+2}]^{1/3}$	E^2 region*	$\gamma = 2$	$[\bar{l}_w\bar{l}_Z\bar{l}_{Z+1}]^{1/3}$	$[\bar{l}_{Z+3}\bar{l}_{Z+4}\bar{l}_{Z+5}]^{1/3}$
	$\gamma = 2$	$[\bar{l}_N\bar{l}_w\bar{l}_Z]^{1/3}$	$[\bar{l}_{Z+1}\bar{l}_{Z+2}\bar{l}_{Z+3}]^{1/3}$		$\gamma = 4$	$[\bar{l}_{Z+1}\bar{l}_{Z+3}]^{1/3}$	$[\bar{l}_{Z+5}\bar{l}_{Z+6}\bar{l}_{Z+7}]^{1/3}$
	$\gamma = 3$	$[\bar{l}_w\bar{l}_Z\bar{l}_{Z+1}]^{1/3}$	$[\bar{l}_{Z+2}\bar{l}_{Z+3}\bar{l}_{Z+4}]^{1/3}$				

* At low degrees of orientation, where the effects are proportional to E^2

More generally we write:

$$D = Al^{-\delta} \tag{12}$$

and rewrite equation (7)

$$\left[\frac{\mathrm{d}(\log Z(t))}{\mathrm{d}t}\right]_{t=0} = \frac{-6A\int l^{p-\delta}n(l)\mathrm{d}l}{\int l^p n(l)\mathrm{d}l} \tag{13}$$

The experimental value value of D as measured from the initial decay is then related to the ratio of two moments and we can write:

$$\bar{D}_{p,\delta} = A\frac{\langle l_{p-\delta}\rangle}{\langle l_p\rangle} \tag{14}$$

It is interesting to rewrite equation (14) so that the average lengths $\bar{l}_q$ appear explicitly:

$$D_{p,\delta} = A\frac{\langle l_{p-\delta}\rangle}{\langle l_{p-\delta+1}\rangle}\,\frac{\langle l_{p-\delta+1}\rangle}{\langle l_{p-\delta+2}\rangle}\cdots\cdots\frac{\langle l_{p-1}\rangle}{\langle l_p\rangle}$$

$$= A\,[\bar{l}_{p-\delta+1}\times\bar{l}_{p-\delta+2}\cdots\cdots\bar{l}_p]^{-1} \tag{15}$$

EXPLICIT EXPRESSION IN THE CASE OF ROD-LIKE PARTICLES AND DISCS

Taking into account the different values of α, β and γ which can be encountered one can build a table of the average sizes associated with the diffusion constants obtained with the different electro-optical effects (see *Table 1*).

It is seen that widely different averages can be reached according to the orientation process and to the electro-optical effect. The exceptional sensitivity of light scattering in an electric field to large particles has already been pointed out by Wippler[12].

RE-EXAMINATION OF PREVIOUSLY PUBLISHED DATA

From equation (15) and *Table 1* it is clearly seen that a given $\bar{D}_p$ is not associated with $\bar{l}_{p+1}$ as indicated by the work of Foweraker and Jennings, but to $[\bar{l}_{p-2}\bar{l}_{p-1}\bar{l}_p]^{1/3}$. This is likely to make an enormous difference as soon as the ratios between successive $\bar{l}_p$ are noticeably different from 1.

(A) In the two cases examined by Foweraker and Jennings p values are assumed to be equal to 1 and 2 so that the experimental values D_w and D_Z should be respectively compared to those calculated from $(\bar{l}_{N-2}\bar{l}_{N-1}\bar{l}_N)^{1/3}$ and $(\bar{l}_{N-1}\bar{l}_N\bar{l}_w)^{1/3}$.

We have found it rather difficult to calculate the averages corresponding to $\bar{l}_{N-1}$ and $\bar{l}_{N-2}$ from the histograms, due to the enormous influence of the smallest class. Indeed taking into account all classes one obtains:

For crocidolite	For attapulgite
$\bar{l}_{N-1} = 0.64\ \mu\mathrm{m}$	$\bar{l}_{N-1} = 0.508\ \mu\mathrm{m}$
$\bar{l}_{N-2} = 0.34\ \mu\mathrm{m}$	$\bar{l}_{N-2} = 0.208\ \mu\mathrm{m}$
$\bar{l}_{N-1}/\bar{l}_{N-2} = 1.88$	$\bar{l}_{N-1}/\bar{l}_{N-2} = 2.44$

while with suppression of the smallest class (2 objects out of 160 or 84) one obtains:

For crocidolite:	For attapulgite:
$\bar{l}_{N-1} = 0.74\ \mu\mathrm{m}$	$\bar{l}_{N-1} = 0.62\ \mu\mathrm{m}$
$\bar{l}_{N-2} = 0.55\ \mu\mathrm{m}$	$\bar{l}_{N-2} = 0.38\ \mu\mathrm{m}$
$\bar{l}_{N-1}/\bar{l}_{N-2} = 1.34$	$\bar{l}_{N-1}/\bar{l}_{N-2} = 1.63$

Taking these values one obtains for the averages associated with D_w and D_Z

$$D_w\text{: }(\bar{l}_{N-2}\bar{l}_{N-1}\bar{l}_N) = \begin{cases} 0.407\ \mu\mathrm{m}^3 \text{ as compared with} \\ \bar{l}_w^{\,3} = 2.2\ \mu\mathrm{m}^3 \text{ for croidolite} \\ 0.176\ \mu\mathrm{m}^3 \text{ as compared with } \bar{l}_w^{\,3} \\ = 0.636 \text{ for attapulgite} \end{cases}$$

$$D_Z : (\bar{l}_{N-1}\bar{l}_N\bar{l}_w) = \begin{cases} 0.96\ \mu\mathrm{m}^3 \text{ as compared with } \bar{l}_Z^{\,3} \\ = 3.94\ \mu\mathrm{m}^3 \text{ for crocidolite} \\ 0.40\ \mu\mathrm{m}^3 \text{ as compared with } l_Z^{\,3} = \\ 1.19\ \mu\mathrm{m}^3 \text{ for attapulgite} \end{cases}$$

There is an extremely large difference. We have recalculated all D averages using the average sizes of *Table 1* for crocidolite and attapulgite and Burgers formula. The results are given in *Table 2*.

From these figures it seems that the experimental values are much closer to D_{Z+1} and D_{Z+2} than D_w and D_Z. If one excludes the possibility of a large swelling of the particle in water solution, there are two possible reasons for this:

(a) Experiments for D_w are supposed to be performed at saturation of orientation of all particles. Since the smallest particles only contribute a very small amount to the birefringence, the electro-optical effect can reach an apparent saturation when the small particles are still far from complete orientation. This would minimize the role of small particles

Table 2 Comparison of calculated and experimental values of the rotatory diffusion constant

	From average sizes (histogram)					Experimental[6]	
	$\langle D_N \rangle$	$\langle D_w \rangle$	$\langle D_{Z+1} \rangle$	$\langle D_{Z+2} \rangle$	$\langle D_{Z+3} \rangle$	$\langle D_w \rangle$	$\langle D_Z \rangle$
Attapulgite	93.5	44	26.8	17.1	11.92	28.4	15.7
Crocidolite	15.30	7.76	4.14	2.52	1.81	4.1	2.35

and shift the apparent D towards higher moments. It would also explain the large value of l_1 derived from the peeling method.

(b) In the Kerr region, the orientation mechanism has been supposed to correspond to $\gamma = 1$ i.e. a polarizability anisotropy proportional to l. It is our experience in colloid particles that the orientation proceeds from an ionic polarizability which, due to the large diameter of the particles, would correspond to the case of weak counterion repulsion and therefore to $\gamma = 3$. In that case the D average would correspond to $p = 4$ i.e. D_{Z+2} in good agreement with our calculations.

Unfortunately the authors have not given the plot corresponding to the peeling method for the Kerr region. It is therefore not possible to calculate the number of particles corresponding to the 'short' and to the 'long' relaxation time under the assumption of $\gamma = 3$ and to check how the recalculated averages, based on a two species distribution, fit with the actual one.

(b) Such a calculation was performed in the first application of the peeling method[1] for the case of the polypeptide PBLG, which is oriented by a permanent moment ($\gamma = 2$, $p = 3$). Unfortunately an error was made in the value of p which was taken equal to 2 with a resulting error both in the average size associated with D and in the number of particles in the species derived from the peeling method.

Reinterpreting the data one has for a sample of molecular weight $M_w = 10^5$ which is likely to be rigid enough to be treated as a rod:

L_0 from $D_{3,3} = 770$ Å
L_1 from peeling method 1020 Å, $n_1 = 0.17$
L_2 from peeling method 600 Å, $n_2 = 0.83$
$L_{N\,calc} = 671$ Å
$L_{w\,calc} = 708$ Å
$L_{Z\,calc} = 757$ Å
$L_{0\,calc} = (L_N L_w L_Z)^{1/3} = 711$ Å

The agreement between L_0 values is satisfactory. Moreover, from M_w and L_w one gets a mass per unit length of 148 in good agreement with the α-helical structure.

ANALYTICAL DISTRIBUTION FUNCTIONS FOR THE CALCULATION OF MOMENTS $\langle l_q \rangle$ WITH $q < 0$

As we have seen that the experiments can give a combination of widely different moments $\langle l_q \rangle$ of the distribution in size including those with $q < 0$, it would be interesting to have a distribution function whose moments are all analytical functions of a few parameters so that the knowledge of a few moments can lead to the prediction of all the others and their eventual comparison with those calculated from the histogram derived from electron micrography. The usual Gaussian or Poisson distribution cannot be used since, in order that $\langle l_q \rangle$ does not diverge for $q < 0$, the probability function should not tend to zero faster than l^{+q}. Two 'classical' distribution functions at least meet this condition:

(a) the log normal distribution

$$n(l) = \frac{1}{(2\pi\sigma)^{1/2}}\,\frac{1}{l}\exp\left[-\frac{1}{2\sigma^2}(\log l - \mu)^2\right] \tag{16}$$

where $\mu = \log l_m$ with l_m the median of the distribution.

(b) the gamma distribution

$$n(l) = \lambda^k e^{-\lambda l} l^{k-1}/\Gamma(k) \tag{17}$$

where k has to be higher than the highest absolute value of q for which $\langle l_{-|q|} \rangle$ has to be calculated.

Moments of these distributions

It is interesting to recall the analytical expressions of $\langle l_q \rangle$ for these distributions and to show how they would fit the distributions reported for crocidolite and attapulgite for which the successive average have been found from the histrograms in the successive ratios:

$l_{Z+2}/l_{Z+1}/l_Z/l_w/l_n/l_{n-1}/l_{n-2} =$

1.08/1.12/1.20/1.32/1.5/2 for crocidolite

1.13/1.15/1.17/1.21/1.25/2.7 for attapulgite

From equations (9), (16) and (17) one obtains for the two distributions:

$$\text{(a)}\quad \langle l_q \rangle = \exp(q\mu + q^2\sigma^2/2) \quad q \gtrless 0$$

one sees that the number-average is given by $\bar{l}_n = l_m \exp(\sigma^2/2)$ and that successive average values are all in the same ratio, $\exp \sigma^2$. This is not too far from the attapulgite distribution but for the very smallest class.

$$\text{(b)}\quad \langle l_q \rangle = \lambda^{-q}\frac{\Gamma(k+q+1)}{\Gamma(k)}$$

for k integer

$$\langle l_q \rangle = \lambda^{-q}\frac{(k+q)!}{(k-1)!}$$

and

$$\bar{l}_{N+m} = \lambda(k+m+1) \qquad m \gtrless 0$$

One sees that the crocidolite distribution is well represented by this distribution with $k = 2$.

CONCLUSION

From our theoretical analysis as well as from the reexamination of published data several conclusions can be drawn.

(1) Well defined average sizes covering a large range of moments, can be derived from electro-optic decay analysis.

(2) The knowledge of the exact mechanism of orientation is needed to decide the type of average involved. Inversely, examination of polydisperse systems can help to draw con-

clusions on the mechanism of orientation without need of well defined fractions to measure the exponent γ.

(3) Work at 'saturating fields' may be very dangerous when the diagnostic of saturation is limited to the appearance of the plateau in the optical effect, with no consideration of its absolute value.

(4) Comparison of birefringence and light scattering at low orientation should be preferred to the comparison of one electro-optical effect as a function of the field as a quantitative diagnostic of polydispersity.

Light scattering in the region $Kl \gg 1$ where the full equation (1) has to be used would also be of value but might be too heavily weighted by the large size tail of the distribution. Work along these lines is now under way.

ACKNOWLEDGEMENTS

HJC thanks Professor H. Benoit for his kind hospitality and the Royal Society for the award of a European fellowship. G. W. thanks Dr S. Sokerov for discussions concerning light scattering in an electric field.

REFERENCES

1 Boeckel, G., Genzling, J. C., Weill, G. and Benoit, H. *J. Chem. Phys.* 1962, **59**, 999
2 Tsuji, K., Watanabe, H. and Yoshioka, K. *Adv. Mol. Relaxation Processes* 1976, **8**, 49
3 Colson, P. and Gaspard, J. P. 'Dynamic Aspects of Conformation Changes in Biological Macromolecules,' (Ed. C. Sadron) Reidel, Dordrecht, Holland, 1973, p. 117
4 Kobayashi, S. *Biopolymers* 1968, **6**, 1491
5 Schweitzer, J. and Jennings, B. R. *Biopolymers* 1972, **11**, 1077
6 Foweraker, A. R. and Jennings, B. R. *Adv. Mol. Relaxation Processes* 1976, **8**, 103
7 Benoit, H. *Ann. Phys.* 1951, **6**, 561
8 Sokerov, S. and Stoimenova, M. V. *J. Colloid Interface Sci.* 1974, **46**, 94
9 Stoimenova, M. V. *J. Colloid Interface Sci.* 1975, **53**, 42
10 Sokerov, S. and Weill, G. to be published
11 Fredericq, E. and Houssier, C. 'Electric dichroism and electric birefringence', Clarendon Press, Oxford, 1973
12 Wippler, C. *J. Chim. Phys.* 1956, **53**, 316
13 Stoylov, S. P. *Collect. Czech. Chem. Commun.* 1966, **31**, 2866
14 Coles, H. J. and Jennings, B. R. *Biopolymers* 1975, **14**, 2567
15 Oosawa, F. 'Polyelectrolytes', Marcel Dekker, New York, 1971
16 Hornick, C. and Weill, G. *Biopolymers* 1971, **10**, 2345
17 Burgers, J. M. *Verh. K.Ned. Akad. Wet. Afd. Natuurk.* 1938, **4**, 113
18 Perrin, F. *J. Phys. Radium* 1934, **5**, 497