

Small-angle X-ray study of air-dried elastoidin using three - and one - dimensional correlation functions obtained from slit - smeared intensity

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Abstract: In the present paper an isotropic sample of air-dried elastoidin has been studied considering it to be a non-ideal two-phase densely packed system after the theories by Vonk [1]. The relevant important physical parameters obtained for the sample are E , the width of the transition layer, D , the average periodicity transverse to the layers, S/V , the specific inner surface ϕ_1 and ϕ_2 , the volume fraction of two phases, i. e. matter and void, \bar{l}_1 and \bar{l}_2 the transversal lengths, \bar{l} , the range of inhomogeneity, l_c , the length of coherence and $2E/D$, the volume fraction of the transition layer. The values of E obtained by two approaches as given by Vonk [1] and Ruland [2] show but a small difference indicating the correctness of the analysis.

Key words: small-angle X-ray scattering (SAXS), elastoidin, correlation functions, width of transition layer.

Introduction

An isotropic sample of air-dried elastoidin was supplied by Ramchandran [3, 4] for small-angle X-ray scattering (SAXS) study. It is an insoluble fibrous protein found in the inner fleshy portion of the fin rays from the shark *carcarinus melanopterus* (Ramchandran [5], page 62) and resembles collagen in many respects [3, 4]. Yet it is distinguished from collagen by its high content of tyrosine, the presence of low but significant amount of cystine and methionine and low content of hydroxyproline. It is prepared after the method due to Damodaran et al. [6].

Elastoidin like collagen is found in the form of long chains containing several amino acids connected by peptide bonds. Three such chains also called strands are folded in left handed helices and are super-coiled in right handed direction to form rod shaped molecules of molecular weight $\sim 300,000$. These rods aggregate in transverse direction resulting in a cylindrical layer structure of diameter varying from 50 Å to 2,000 Å (Ramchandran [5], page 185). It is worth while mentioning here that, as in fibrous protein the scattering particles of elastoidin are taken as arranged in layers.

As the important extra-cellular component of the mesodermal tissues, collagen occupies a pivotal position in the molecular architecture of higher animals which enables it to be the main agent controlling the

distribution of both externally and internally applied forces within the organism. As elastoidin is similar to collagen and comes under macromolecular systems, small-angle X-ray scattering (SAXS) study has been undertaken to throw some light on its macromolecular structure.

In general an ideal two-phase system is defined as one in which the electron density difference between the two phases changes over a sharp boundary. For SAXS studies of such densely packed systems equations derived by Porod [7, 8] (known as the Porod's laws) and Debye, Anderson and Brumberger [9] are commonly used. In reality, between the crystalline and void phases, there exists a region known as transition layer of width E over which the electron density changes continuously (Vonc [1] and Ruland [2]). Such a system is characterised as a non-ideal two-phase system. The ideal two-phase system may be considered as a hard core model and the non-ideal two-phase system as one in which the hard core is coated with a soft sheath with continuously varying electron density.

Experimental

The slit-smeared SAXS data of air-dried elastoidin in arbitrary units have been taken from the Ph. D. dissertation of one the co-authors Misra [10] and they have been shown in figure 1 with extrapolated points marked as ∇ and the background intensities as \bar{I}_{bg} .

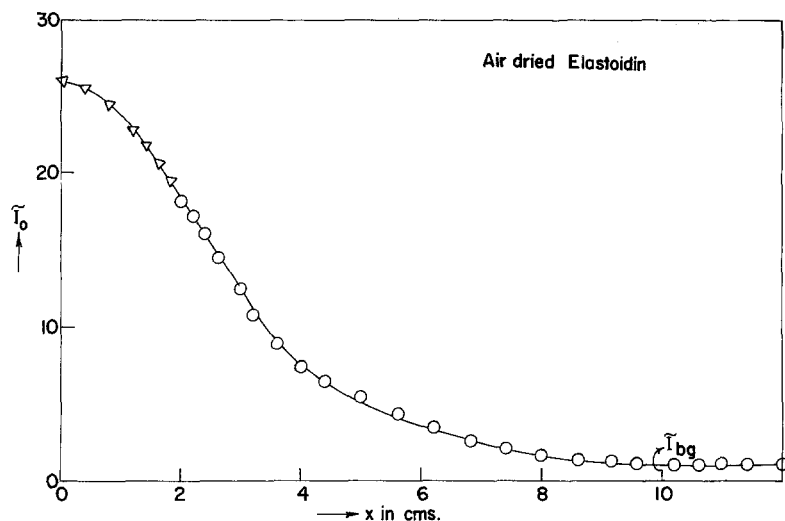


Fig. 1. Smearedout scattering curve

A line focus Matchlett A-2 X-ray diffraction tube with a Cu target was run at 30 KV and 20 mA constant power level with the line voltage stabilised by a servo electric stabiliser. The SAXS camera after Kratky [11], capable of recording the scattering down to an angle of 1.3×10^{-4} radian corresponding to a Bragg value of 12,000 Å, was used. The collimating system was a slit of width 120 μ and was free from parasitic scattering. The sample was taken in a Mark capillary tube of diameter 0.2 cm with negligible scattering. To avoid air scattering, the space in between the film and the sample was evacuated to 5×10^{-3} Tor. The selection of CuK_α radiation (wave length $\lambda = 1.54$ Å) was done by a bent crystal monochromator after Johansson [12] and Guinier [13]. For recording the SAXS pattern due to the sample, the photographic method was employed following the technique of Kratky and Sekora [14]. Assuming the intensity to be proportional to the time of exposures, a single curve of scattered intensities was drawn on microphotometering each partial scattering patch and bringing all the intensity values to the same relative time scale. The transformation ratio of the microphotometer 'p' was 100 and the film-sample distance 'a' was kept at 23.4 cms.

The theory

In the following theory 's' is the co-ordinate in reciprocal or Fourier space given by the relation $s = 2\theta/\lambda$, 2θ being the scattering angle and x , the microphotometered film co-ordinate, is given by the relation $x = 2ap\theta$. So s can be written as $s = x/\lambda ap$. The centre of the primary beam is taken as origin.

For the general two-phase systems having isotropic structures, as shown by Vonk [1], the following relation holds good:

$$16\pi^3 \int s^4 I_a(s) ds = \int |\text{grad } \eta|^2 dv_r. \quad (1)$$

Here $I_a(s)$ is the desmeared intensity in absolute units and η is the deviation of electron density of the sample at any point from the mean value.

The above equation can be regarded as parallel to the well known relation

$$4\pi \int s^2 I_a(s) ds = \int \eta^2 dv_r. \quad (2)$$

If the absolute intensities are not available the ratio of (1) and (2) can be used and denoted as R which is a very important parameter, characteristic of the structure. So R is given by

$$\begin{aligned} R &= \frac{\langle |\text{grad } \eta|^2 \rangle}{\langle \eta^2 \rangle} = 4\pi^2 \frac{\int s^4 I(s) ds}{\int s^2 I(s) ds} = \\ &= 6\pi^2 \frac{\int s^3 \tilde{I}(s) ds}{\int s \tilde{I}(s) ds}. \end{aligned} \quad (3)$$

Here $I(s)$ and $\tilde{I}(s)$ are desmeared and smeared-out intensities respectively in arbitrary units. In an ideal two-phase structure the gradient at the phase boundary is infinity and consequently R goes to infinity. On the otherhand if R is finite the electron density changes from phase I to phase II continuously over a transition layer of width E . The above equation when expressed in terms of x gives

$$R = \frac{3}{2} \left(\frac{2\pi}{\lambda ap} \right)^2 \frac{\int x^3 \tilde{I}(x) dx}{\int x \tilde{I}(x) dx}. \quad (4)$$

The value of E can be obtained from $C(r)$, the three-dimensional correlation function of the sample normalised to unity at the origin in real space. The relation given by Vonk [1] is

$$E = -\frac{4}{R} (dC(r)/dr)_{r=E}. \quad (5)$$

Therefore it is necessary to evaluate the values of $C(r)$ at various values of r in real space. The expression for $C(r)$ from the smeared-out intensities is given, after Mering and Tchoubar [15] by the relation

$$C(r) = \int s \tilde{I}(s) J_0(2\pi rs) ds / \int s \tilde{I}(s) ds$$

Here J_0 is the Bessel function of zero order of the first kind. In the above expression the three-dimensional correlation function, $C(r)$ is determined from the smeared-out intensity $\tilde{I}(s)$ where the slit correction for infinite height given by the relation

$$\tilde{I} = \int_{-\infty}^{\infty} I(\sqrt{s^2 + t^2}) dt$$

is incorporated [16] and is contained in the Bessel function J_0 . Here 't' is an arbitrary variable representing the slit height. In terms of the variable x , $C(r)$ can be written as

$$C(r) = \int x \tilde{I}(x) J_0(2\pi rx/\lambda ap) dx / \int x \tilde{I}(x) dx. \quad (6)$$

For a layer structure, Kortleve and Vonk [17] have shown the use of one-dimensional correlation function $C_1(y)$. The expression for $C_1(y)$ is given by Mering and Tchoubar [15] as

$$C_1(y) = \frac{\int s \tilde{I}(s) [J_0(z) - zJ_1(z)] ds}{\int s \tilde{I}(s) ds}$$

Here $z = 2\pi sy$ and J_1 is the Bessel function of first order of the first kind. When transformed to x variable the above relation becomes

$$C_1(y) = \frac{\int x \tilde{I}(x) [J_0(z) - zJ_1(z)] dx}{\int x \tilde{I}(x) dx} \quad (7)$$

where $z = 2\pi xy/\lambda ap$.

According to Vonk [1] the position of the first subsidiary maximum in the one-dimensional correlation function gives the value of the average periodicity D transverse to the layers.

Using the following relation

$$[dC_1(y)/dy]_{y>E} = -\frac{1}{D} \Delta\eta^2 / \langle \eta^2 \rangle \quad (8)$$

given by Vonk [1] the value of $\Delta\eta^2 / \langle \eta^2 \rangle$ can be computed, where $\Delta\eta$ is the electron density difference be-

tween the two phases. Here the slope is taken at a point y greater than E .

The specific inner surface, S/V , defined as the phase boundary per unit volume of the dispersed phase, is given for a layer structure, by Vonk [1] as

$$S/V = 2/D. \quad (9)$$

For non-ideal two-phase structures the following relation holds good (Vonk [1])

$$\langle \eta^2 \rangle / \Delta\eta^2 = \left(\phi_1 \phi_2 - \frac{E}{6} \frac{S}{V} \right) \quad (10)$$

where ϕ_1 and ϕ_2 are the volume fraction of the two phases, matter and void respectively. For this purpose the phase boundary is chosen at the middle of the transition layer. Taking the sum of the volume fractions of two phases to be unity, the above relation can be utilised to get the values of ϕ_1 and ϕ_2 .

In an irregular two-phase system if arrows are shot in all possible directions the average intersection lengths of the arrows in the two phases are called the transversal lengths and are denoted by \bar{l}_1 and \bar{l}_2 . They are given by the relation (Mittelbach and Porod [18])

$$\bar{l}_1 = 4\phi_1 V/S \text{ and } \bar{l}_2 = 4\phi_2 V/S. \quad (11)$$

The range of inhomogeneity \bar{l}_r is given by Mittelbach and Porod [18] as

$$\frac{1}{\bar{l}_r} = \frac{1}{\bar{l}_1} + \frac{1}{\bar{l}_2} \quad (12)$$

and is similar to the concept of reduced mass in mechanics.

The length of coherence for a specimen is given by the relation (Mittelbach and Porod [18])

$$l_c = 2 \int C(r) dr. \quad (13)$$

A second method for the estimation of the value of E is given by Ruland [2]. The functional relation of $\tilde{I}(s)$ with s at the tail end of the SAXS pattern for non-ideal two-phase system is given by

$$\tilde{I}(s \rightarrow \infty) = \frac{\pi c}{2} (1/s^3 - 2\pi^2 E^2/3s)$$

where c is a proportionality constant and when $E = 0$ for an ideal two-phase structure the above relation

reduces to the Porod's law. On changing to x variable the above equation can be put in the form

$$\tilde{I}(x \rightarrow \infty) \cdot x = \frac{\pi c}{2} (\lambda a p)^3 \cdot x^{-2} - \frac{\pi^3 c}{3} (\lambda a p) E^2. \quad (14)$$

The value of E can be calculated from the plot of $\tilde{I}(x \rightarrow \infty) \cdot x$ versus x^{-2} known as the Ruland plot.

Background correction

In every SAXS experiment it is observed that always a continuous background scattering is superimposed over the SAXS pattern of the sample. Both the methods of estimation of the width of transition layer yield results that are sensitive to the systematic errors in the tail region of the SAXS curve. The correlation function at the origin are also sensitive to the errors in the tail region of the SAXS curve (Vonk [1]). Therefore special attention must be given to separate the SAXS intensities $\tilde{I}(s)$ from the continuous background scattering $\tilde{I}_{bg}(s)$ (Vonk [1]). As reported by Kortleve et al. [19] the continuous background in some pattern shows an upward trend at large values of s after a minimum is attained in the observed SAXS curve and in such cases the experimentally observed data at the tail end can be fitted to an equation of the type

$$\tilde{I}_{bg}(s) = a + bs^n \quad (15)$$

where a and b are constants and n is an even integer. However it was shown by Konrad and Zachmann [20] that $\tilde{I}_{bg}(s)$ remains constant in the region where $\tilde{I}(s)$ contributes appreciably. For both the assumptions corresponding to the background intensity the value of E when calculated following the method of Ruland [2] shows but relatively small difference. So one is justified in deducing a constant background intensity corresponding to the minimum in the SAXS pattern. This background corrected SAXS intensities should be used in subsequent analysis.

Calculation and results

Prior to proceeding further, five initial intensity values were fitted to the Gauss curve (Vonk [21])

$$\tilde{I}(x \rightarrow 0) = p \cdot \exp(-qx^2)$$

by least square technique and the values of constants p and q were obtained as equal to 24.8 and 0.1 respectively. Taking these values of p and q , the scattering

curve was extrapolated to $x = 0$. It may be mentioned here that the method of extrapolation has little effect on the relevant part of the correlation function, neither the position nor the height of the first maximum of the one-dimensional correlation function is much affected.

The two integrals in equation (4) were determined by numerical integration employing the technique of Simpson's one third rule and the value of R was found out to be $7.9 \times 10^{-5} \text{ \AA}^{-2}$. This shows that the gradient of electron density at the phase boundary is finite suggesting the sample to be a non-ideal two-phase system.

Using the relation (6) the three-dimensional correlation function, $C(r)$, was computed for various values of r and is shown in figure 2 in a magnified form beyond $r = 800 \text{ \AA}$. The slopes of $C(r)$ at different points were computed by numerical differentiation with constant difference of 1 \AA interval taking five consecutive points including the point at r where the slope is found out by central difference method. The values of $-\frac{4}{R} \frac{dC(r)}{dr}$ versus r have been plotted in figure 3. In this figure, a straight line equidistant from both the axes has been drawn and the point of intersection of this line

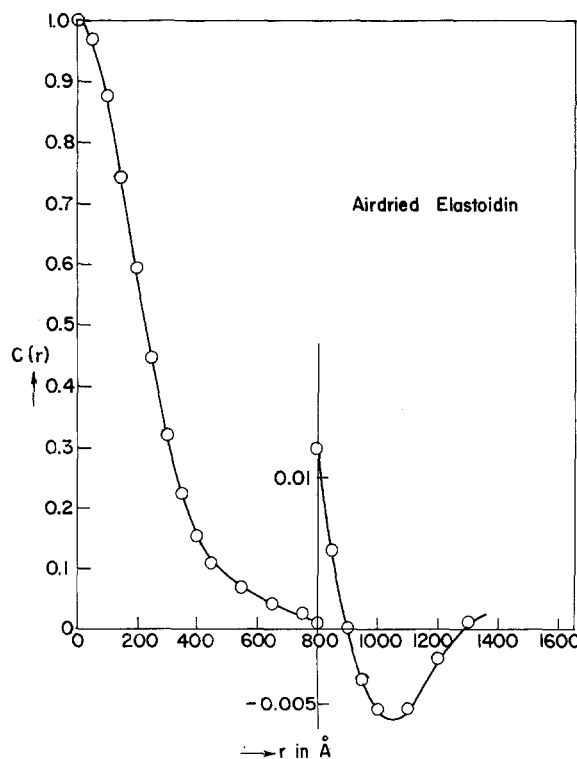


Fig. 2. The curve showing the values of three-dimensional correlation function $C(r)$ against ' r ' values

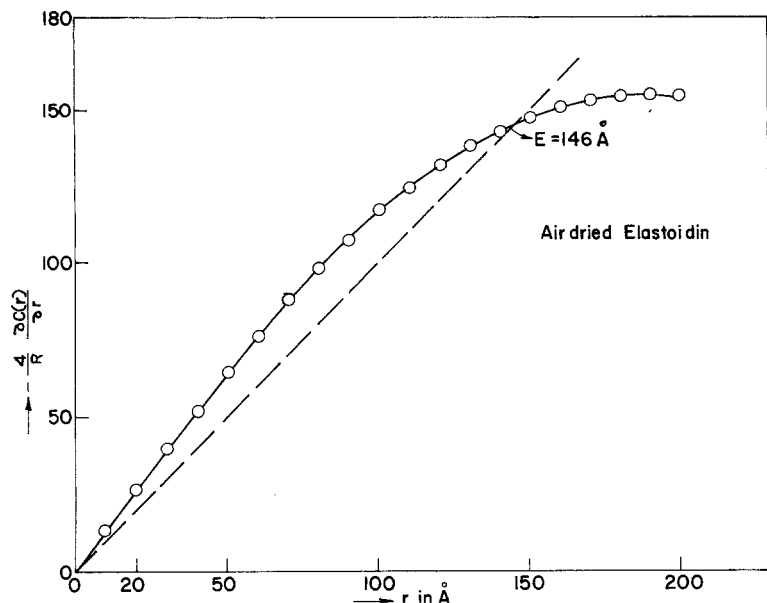


Fig. 3. The curve showing the various values of $-\frac{4}{R} \frac{\partial C(r)}{\partial r}$ against 'r' values

with the curve gives the value of E referred to as E_1 following the relation (5) and it comes out to be 146 Å.

Following the relation (7) the one-dimensional correlation function $C_1(y)$ was calculated for various values of y and is plotted in figure 4 against various values of y . Beyond $y = 1100$ Å both the axes have been shown in magnified scale. The values of D , the average distance of periodicity was found out to be 1192 Å given by the position of the first subsidiary maximum of the same curve.

The value of S/V was obtained as equal to $1.68 \times 10^{-3} \text{ Å}^{-1}$ utilising equation (9).

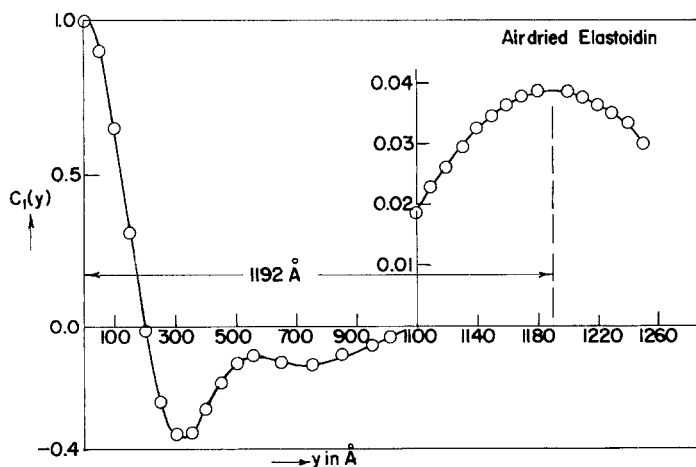


Fig. 4. The curve showing the values of one-dimensional correlation function $C_1(y)$ against 'y' values

The values of $\frac{dC_1(y)}{dy}$ were calculated at various points. It was seen that the slopes came out to be constant equal to 6.96×10^{-3} for various values of $y > E$. Using the above constant the value of $\Delta\eta^2/\langle\eta^2\rangle$ was found to be 8.3 from relation (8).

The value of $(\phi_1\phi_2)$ was found to be 0.13 after relation (10). Taking $\phi_1 + \phi_2 = 1$ we got the values of ϕ_1 and ϕ_2 as equal to 0.85 and 0.15 respectively.

Following the relation (11), the transversal lengths were determined as

$$\bar{l}_1 = 2027 \text{ Å} \text{ and } \bar{l}_2 = 357 \text{ Å}.$$

According to the relation (12) the range of inhomogeneity was found to be

$$\bar{l}_r = 304 \text{ Å}.$$

The length of coherence was found from relation (13) to be

$$l_c = 527 \text{ Å}.$$

The value of $\frac{2E}{D}$, the volume fraction of the transition layer, was computed to be 24.5%.

The Ruland plot of $\bar{l}(x \rightarrow \infty) \cdot x$ versus x^{-2} , shown in figure 5, gives a straight line at the tail end of the scattering curve. The slope and intercept of this line came out to be equal to 881 and -9 respectively giving the

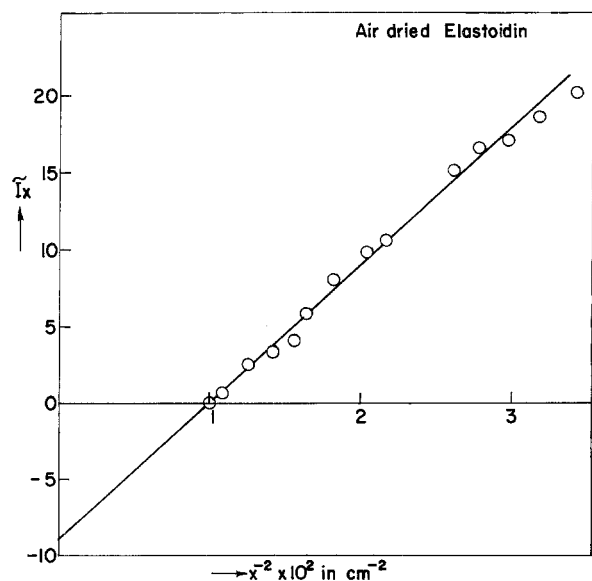


Fig. 5. The plot $\tilde{I}x$ versus $\frac{1}{x^2}$ values

value of the thickness of the transition layer, E_2 , by Ruland [2] method as equal to 142 Å. The standard deviation of intensities $\sigma(I)$ at the tail end of the SAXS pattern was calculated to be equal to 0.12.

Conclusion and discussion

It is evident from equation (14) that for an ideal two-phase system the intercept of the Ruland plot vanishes leading to the application of Porod's law.

When this intercept becomes negative the system belongs to a non-ideal two-phase systems and its magnitude provides a measure of deviation from the Porod's law. Whenever it becomes positive two possibilities may arise, i.e., either the system does not belong to a two-phase system or the dimensions of the scattering particles are too small to be studied by SAXS method.

In this study, the finite negative intercept of the Ruland plot in figure 5 and the finite value of R suggest that the sample of air-dried elastoidin belongs to a non-ideal two-phase structure. The small difference in the values of $E_1 = 146$ Å and $E_2 = 142$ Å as determined by the two methods shows the correctness of the analysis.

The values of D , the periodicity transverse to the layers, is 1192 Å and is well within the range of 50–2000 Å as mentioned in the introduction section for collagen.

The values of $S/V = 1.68 \times 10^{-3} \text{ Å}^{-1}$, $\phi_1 = 0.85$, $\phi_2 = 0.15$, $\bar{l}_1 = 2027 \text{ Å}$, $\bar{l}_2 = 357 \text{ Å}$, $l_r = 304 \text{ Å}$, $l_c = 527 \text{ Å}$ and $\frac{2E}{D} = 24.5\%$ throw some light on the structural aspect of elastoidin, a sample similar to collagen.

The values of $C(r)$ initially remains positive and it goes to zero for a dilute system at large value of r while in a densely packed system it becomes negative at large values of r (Guinier and Fournet [22]). But in this case of non-ideal two-phase system the $C(r)$ function is seen to oscillate (fig. 2) at large values of r .

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