

Collagen dehydration

Richard G. Haverkamp^{a,*}, Katie H. Sizeland^b, Hannah C. Wells^a, Christina Kamma-Lorger^c

^a School of Engineering and Advanced Technology, Massey University, Palmerston North 4442, New Zealand

^b ANSTO, New Illawarra Road, Lucas Heights, NSW 2234, Australia

^c BioSAXS, ANSTO Australian Synchrotron, 800 Blackburn Rd, Clayton, 3168, VIC, Australia

ARTICLE INFO

Keywords:

Collagen

Small angle X-ray scattering

Dehydration

ABSTRACT

Type I collagen is a ubiquitous structural protein in animal tissues. It is normally present in a hydrated form. However, collagen is very dependent on associated water for its mechanical properties. In skin, where type I collagen is dominant, there is a longstanding concern that the skin and therefore collagen may partially dry out and result in structural degradation. Here we show that dehydration of type I collagen fibrils, using 2-propanol, results in a two-stage dehydration process. Initially, the fibrils do not change length, i.e. the D-period remains constant, but shrinkage occurs within the fibrils by an increase in the gap region and a decrease in the overlap region within a D-band and a shortening of the helical turn distance and fibril diameter. Only with further dehydration does the length of the collagen fibril decrease (a decrease in D-period). This mechanism explains why collagen materials are resistant to gross structural change in the early stages of dehydration and shows why they may then suffer from sudden external shrinkage with further dehydration.

1. Introduction

Type I collagen forms fibrils composed of tropocollagen units. The fibrils have a banded structure with regions of overlaps and gaps, where the overlap contains all the tropocollagen “molecules” in a fibril and the gap contains 4/5 of the molecules. By a curious quirk of the structure of type I collagen fibrils, the gap length is almost the same as the overlap length when collagen is hydrated. However, the structure of collagen fibrils changes with hydration. With dehydration fibrils are known to shrink in length (D-period) [1–3] and width (fibril diameter) and the tropocollagen packs closer together [4–7]. The change in length shows a large unexplained apparent anomaly. That is, with initial dehydration the D-period changes very little, until a critical level of dehydration is achieved when the D-period then starts to shrink considerably [7].

Collagen is the major structure-forming material for animals. Type I collagen is the major component of skin where it is the physical barrier between the environment and the body fluids. The skin has to resist water loss. The collagen of the dermis can be subjected to partial dehydration, which can affect function and appearance, and this drives a large component of the cosmetics industry. An understanding of how dehydration of collagen is evident in the collagen fibril and the physical changes that take place in each stage of dehydration may be useful in the biological context, but also in materials, both industrial and medical,

manufactured from collagen.

This work seeks to investigate the apparent anomaly in the change in D-period with dehydration and address more fully the molecular and nanoscale changes taking place with dehydration. We report experimental evidence of the changes in type I collagen fibril structure when dehydrated with 2-propanol–water solutions and provide an explanation of the processes involved in collagen fibril dehydration.

2. Materials and methods

2.1. Materials

A native collagen material in the form of decellularised calf skin, was used, commercially available as surgical scaffold material, SurgiMend, supplied by TEI Biosciences, Waltham, MA, USA (now Integra Lifesciences). This material has fat, glycosaminoglycans and cellular material removed leaving mainly collagen in a native, non-denatured form. The use of this available material enables this experimental work to be readily reproduced. Propan-2-ol was Univar grade from Ajax Finechem, Sydney, Australia.

* Corresponding author.

E-mail address: r.haverkamp@massey.ac.nz (R.G. Haverkamp).

<https://doi.org/10.1016/j.ijbiomac.2022.06.180>

Received 26 April 2022; Accepted 27 June 2022

Available online 4 July 2022

0141-8130/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

2.2. SAXS/WAXS

Diffraction patterns were recorded on the Australian Synchrotron SAXS/WAXS beamline, using a high-intensity undulator source. Energy resolution of 10^{-4} was obtained from a cryo-cooled Si(111) double-crystal monochromator and the beam size (FWHM focused at the sample) was $250 \times 80 \mu\text{m}$, with a total photon flux of about $2 \times 10^{12} \text{ph.s}^{-1}$ using a Pilatus 1 M detector with an active area of $170 \times 170 \text{mm}$. Two experimental setups were used. For low q measurements (SAXS) a sample-to-detector distance of 3371 mm was used with an X-ray energy of 12 keV. For high q measurements (WAXS), the sample-to-detector distance was 900 mm with an X-ray energy of 15.3 keV. Exposure time for diffraction patterns was 1–5 s and initial processing of the data was with Scatterbrain software [8].

2.3. Fourier transform and data fitting

IgorPro software by Wavemetrics, Portland, OR, USA, was used for Fourier transformation of the X-ray scattering data and fitting to models. The collagen model consisted of a 100 cycle square wave stepping from an intensity of 0.8 to 1.0 (which represents 100 D-periods with a step function between gap and overlap regions at a density ratio of 4:5), and 10,000 points to the function (i.e. 100 points per D-period). The duty cycle of the square wave (representing the proportion of the total D-period that is either gap or overlap) was varied for a best fit to the diffraction pattern. For WAXS measurements where the position of the scattering peaks was required, the data were fitted to a gaussian curve using the peak fit function in IgorPro.

3. Results

3.1. SAXS patterns

SAXS patterns for representative samples are shown in Fig. 1. Diffraction bands due to scattering from the D-period of collagen are clearly visible. From these scattering patterns, the scattering intensity can be integrated over all azimuthal angles to give a 1-D plot of intensity (I) versus the scattering vector (q) (Fig. 2) sacrificing information on the

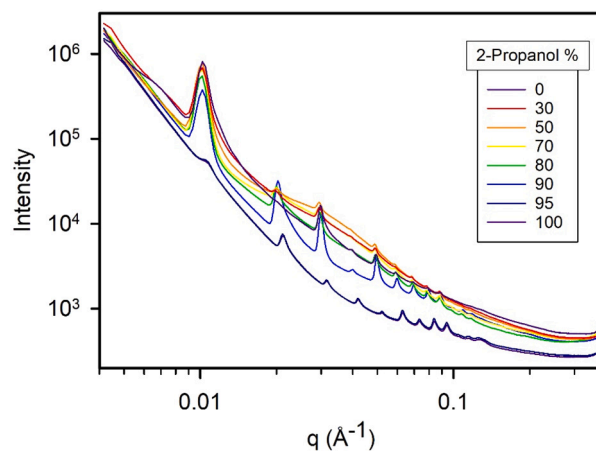


Fig. 2. SAXS azimuthally integrated intensity plots for collagen in 0–100 % 2-propanol–water mixtures.

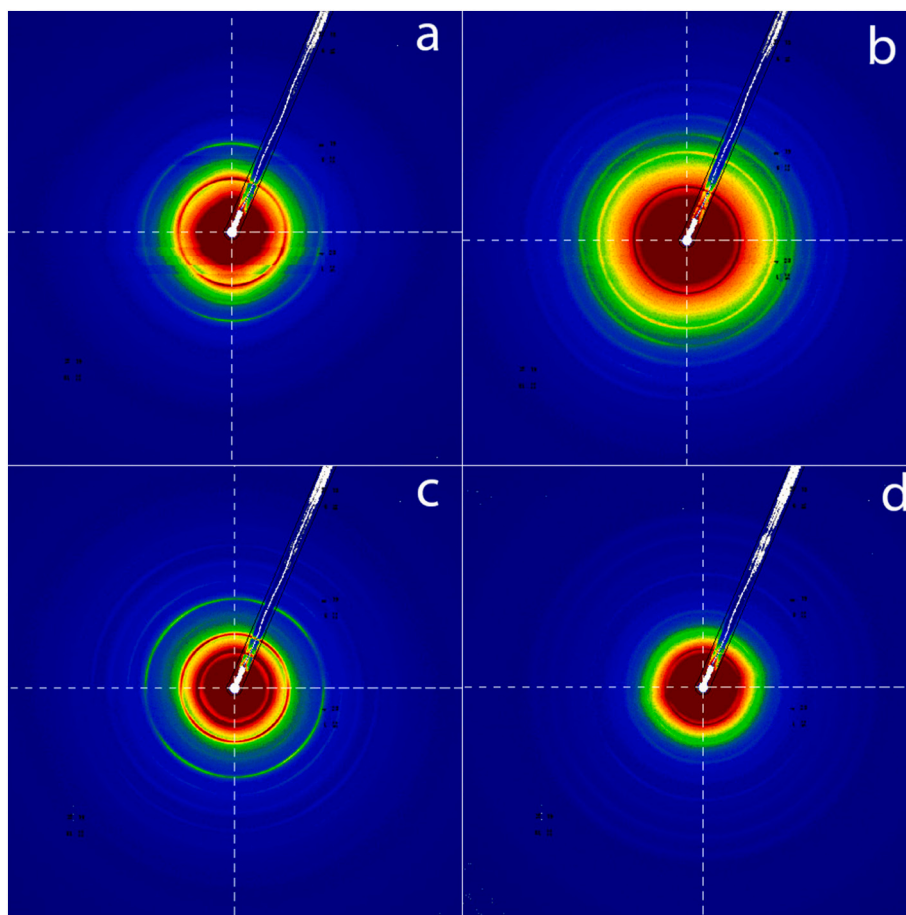


Fig. 1. SAXS patterns (displaying a q range of $0.004\text{--}0.4 \text{Å}^{-1}$) for a) 0 % 2-propanol, b) 80 %, c) 90, d) 100 %.

collagen fibril orientation, which is not relevant to this work.

3.2. D-period change with hydration

There is a shift in the position of the diffraction peaks with 2-propanol concentration. The shift can be measured by determining the position of a diffraction peak of any order. As collagen is dehydrated to different degrees by 2-propanol–water mixtures in different proportions the D-period decreases. At first the D-period appears to increase slightly with dehydration then drops dramatically above 90 % concentration of 2-propanol (Fig. 3). However, the length of the D-period obtained from the different diffraction orders varies.

3.3. Gap-overlap change (from odd/even peak areas and FFT simulation)

The D-banding of collagen fibrils results from areas of overlap of collagen molecules (containing 5n molecules) and so called gap regions (containing 4n molecules) (Fig. 4). This is clearly visible in transmission or high resolution scanning electron microscopy images or in atomic force microscopy images. From the X-ray diffraction data the length of both the gap region and the overlap region can be found (summing to the total D-period). The X-ray scattering pattern results from the combination of the scattering from all the atoms in the structure being studied. Diffraction peaks occur in a crystalline material where there is long range order because at the position of the detector of these diffraction peaks the X-ray scattering from the atoms arrives in phase. A Fourier transform of the three-dimensional atom spacing in a crystal will provide a simulation of the diffraction pattern that would be obtained. Collagen fibrils can be considered a one-dimensional crystal, in other words they have a regular long-range order running the length of the fibril but not so much at right angles to the fibril's long axis, therefore X-ray scattering from this long axis order results in diffraction peaks. For collagen then, the major component of the diffraction pattern can be

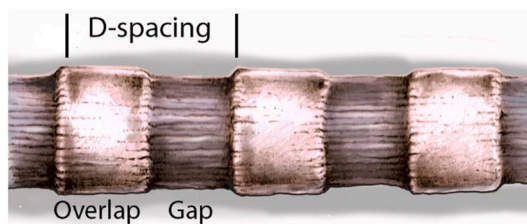


Fig. 4. Sketch of a collagen fibril showing the D-period, gap and overlap regions.

obtained by a one-dimensional Fourier transform of the collagen structure, where by collagen structure we mean at the scale of the D-banding. The density ratio between the gap and the overlap is around 4:5 (i.e. the gap has 80 % of the electron density of the overlap). The transition between the gap and the overlap can be modelled adequately by a step function [9]. In order to do the reverse, and calculate the structure from the diffraction pattern, one might imagine that an inverse Fourier transform would provide the solution. However, X-ray diffraction only provides information on the diffracted X-ray intensity but not on the phase, and of course out of phase X-rays (that have travelled a path length that is different by half an X-ray wavelength) will cancel out, therefore there is incomplete information to make this direct calculation. The solution to this problem is to take a possible structure, simulate the diffraction pattern by Fourier transform, then iteratively adjust the structure until the simulated diffraction pattern fits the recorded data.

The 1-D scattering data for collagen over the q range covering diffraction peaks of order 1–9 was fitted for the ratio of intensities for each odd/even pair to the ratio of intensities for that pair from the FFT of a model for collagen with a varying gap and overlap ratio (Fig. 5). From these fits to diffraction patterns gap-overlap was determined for collagen in each 2-propanol–water mixture (Fig. 6a). There is considerable variation between the gap-overlap determined at different adjacent odd-even order peak pairs.

An alternative method to estimate the gap-overlap from odd-even peak intensity ratios is that described by Eq. (1) [1,3,10].

$$\frac{I_{n+1}}{I} = \left(\frac{n}{n+1}\right)^2 \left[\frac{\sin(n+1)\pi\frac{O}{D}}{\sin(n\pi\frac{O}{D})} \right]^2 \quad (1)$$

where I is the intensity, n is the peak order, O is the overlap proportion and, D is the D-period.

This was also used to calculate the gap-overlap from the same data

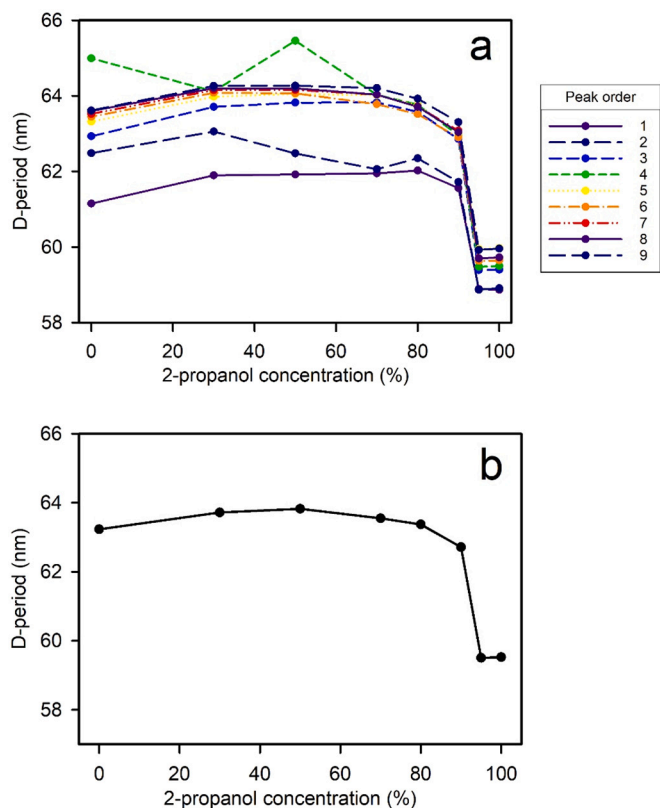


Fig. 3. Collagen D-period in 0–100 % 2-propanol measured using the position of 1–9th order diffraction peaks.

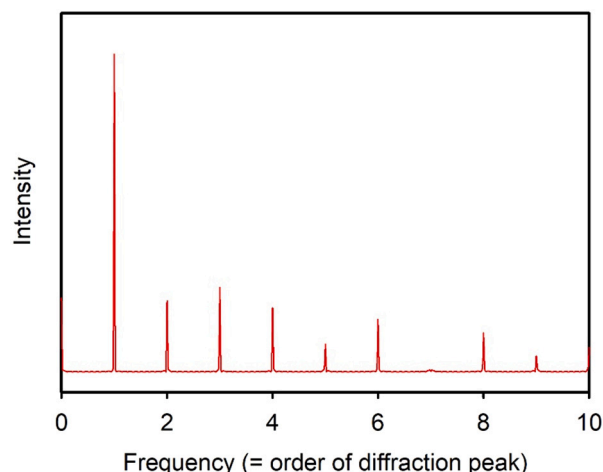


Fig. 5. Example FFT of a model of collagen (with overlap length = 0.429).

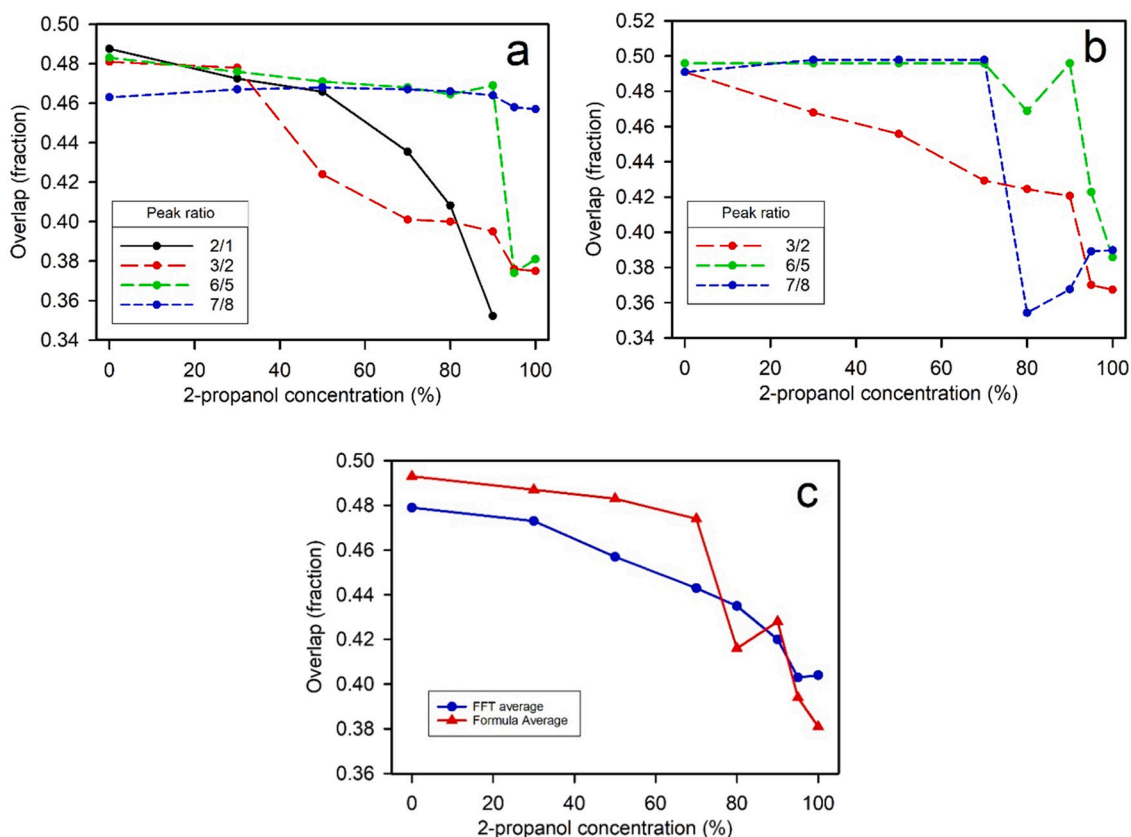


Fig. 6. Calculated overlap fraction (of the D-period) a) using the FFT method, b) using the method of [1,3,10], c) the average of peak pairs for each method.

(Fig. 6b), although it was noted that “this approach is a very crude approximation” [3]. There is considerable variation between the gap-overlap calculated for different pairs estimated by this method.

Taking an average of the fits for the adjacent odd-even pairs a trend of decreasing overlap is seen from low 2-propanol concentrations through to 100 % 2-propanol (Fig. 6c). An improvement on this method would be to fit the full FFT to all 9 recorded diffraction peaks, with the gap-overlap as the variable.

3.4. WAXS diffraction patterns

WAXS patterns for representative samples are shown in Fig. 7. This region covers the dimensions of the tropocollagen helical turn and the tropocollagen molecular diameter. From these scattering patterns, the scattering intensity can be integrated over all azimuthal angles to give a 1-D plot of intensity (I) versus the scattering vector (q) (Fig. 8).

3.5. Helical turn change

From the WAXS patterns, the tropocollagen helical turn distance, the distance measured along the length of the tropocollagen molecule for one full helical rotation, can be measured [6]. This is the atomic distance given by the small diffraction peak at q of around 2.2 \AA^{-1} . This length decreases as the 2-propanol concentration increases, with a sharp decrease at 100 % 2-propanol (Fig. 9).

3.6. Intermolecular spacing

The intermolecular packing was determined from the SAXS peaks in the q range $0.2\text{--}0.7 \text{ \AA}^{-1}$. The intermolecular distance (center to center) decreases from 15.4 \AA with water to 11.0 \AA in 100 % 2-propanol (Fig. 10).

3.7. Derived measurement – relative molecule length

The collagen molecule length is made up of four complete D-periods plus one overlap region. The length changes with dehydration and this can be calculated from the measured D-period and the measured overlap length at each stage of dehydration (2-propanol concentration) (Fig. 11) although the sum of these is dominated by the D-period. While the shape of this plot appears superficially similar to the change in length of the helical turn with dehydration, the relative magnitude of the change is quite different, with a much larger relative change in the molecule length with dehydration (c. $10\times$ larger). Also the molecular length initially increases in the early stages of dehydration before sharply shrinking, unlike the helical rise per residue which decreases throughout dehydration.

3.8. Solvent scattering

A large and broad scattering peak is seen in the WAXS patterns at a q of around $2.0\text{--}1.5 \text{ \AA}^{-1}$ for the water 2-propanol range with a second peak at about 0.8 \AA^{-1} for the higher concentrations of 2-propanol (Fig. 8). This is due to the solvent mixture present in the collagen material [11,12]. The peak changes from a broad peak for water at around 3.3 \AA to a less broad but double peak for 2-propanol with the dominant peak at 4.5 \AA (Fig. 12). This has been well characterised elsewhere [12] but is worth noting here as this falls in the range of interest for collagen structural features.

4. Discussion

There is a range of structural changes that take place in collagen at the molecular and fibrillar (nano) scale as collagen is dehydrated. These involve shrinkage, but at different levels of hierarchy. We can understand what these changes are and how the cascade of shrinkage occurs.

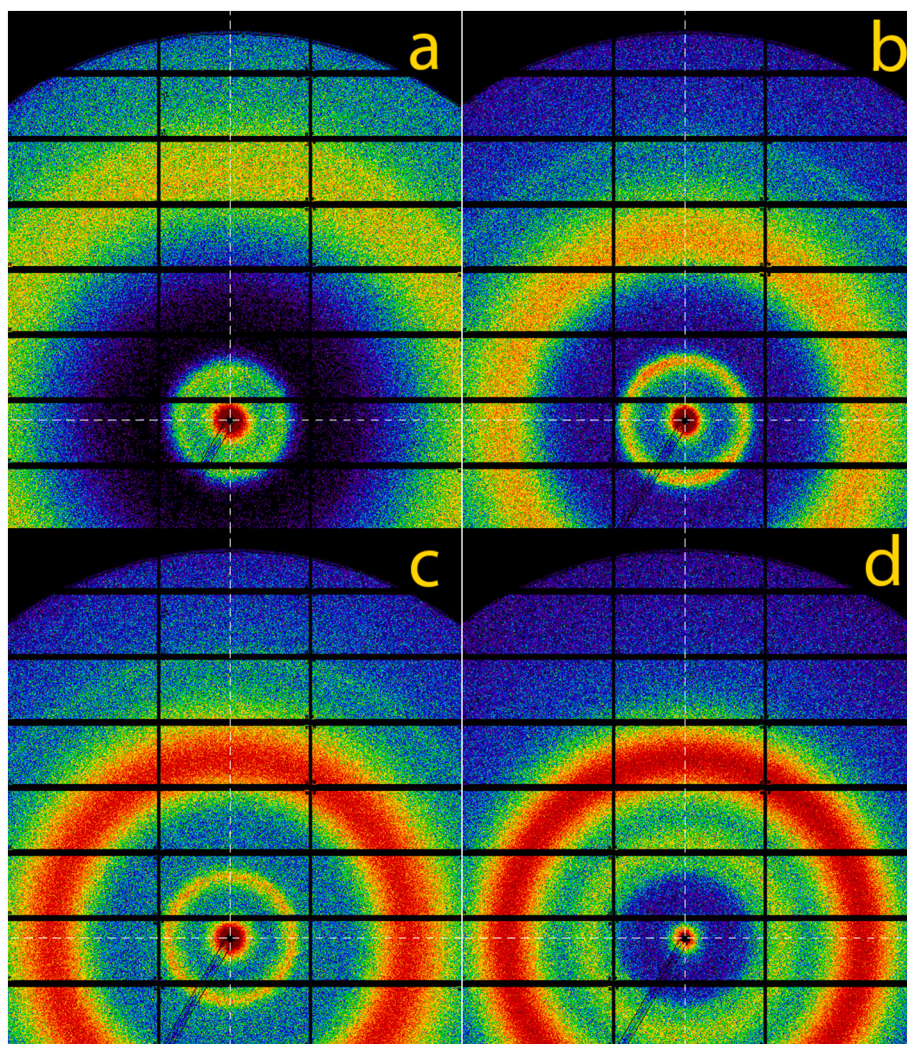


Fig. 7. WAXS patterns (displaying a q range of $0.03\text{--}3.0 \text{ \AA}^{-1}$) for a) 0 % 2-propanol, b) 80 %, c) 90 %, d) 100 %.

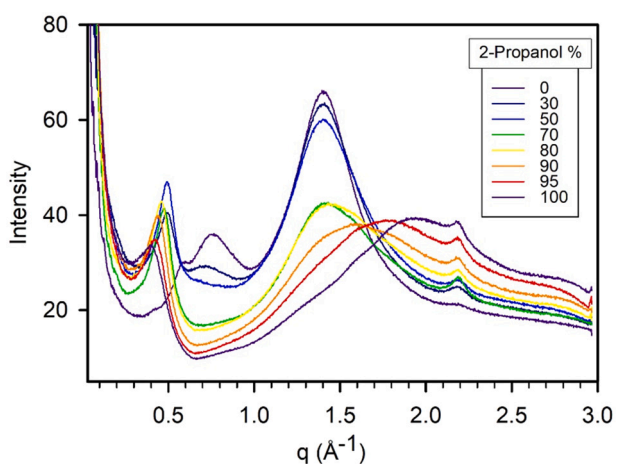


Fig. 8. WAXS azimuthally integrated intensity plots for collagen in 0–100 % 2-propanol.

It has previously been noted that the D-period slightly increases with dehydration before it decreases dramatically [7]. This behaviour seemed at odds with other measured changes in collagen such as the tropocollagen molecular diameter which shrinks across the whole range of

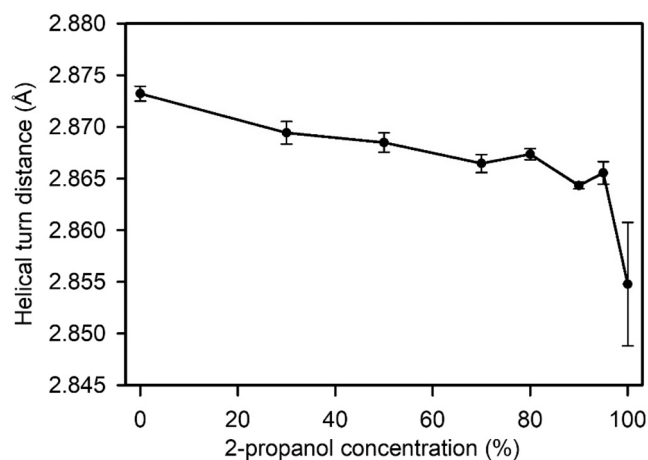


Fig. 9. Helical turn measured by WAXS for collagen in 0–100 % 2-propanol. Error bars are one standard deviation.

dehydration.

However, the collagen molecules do in fact shrink both in length and diameter throughout the range of dehydration. The shrinkage in length is initially taken up by the gap region of the collagen fibril increasing

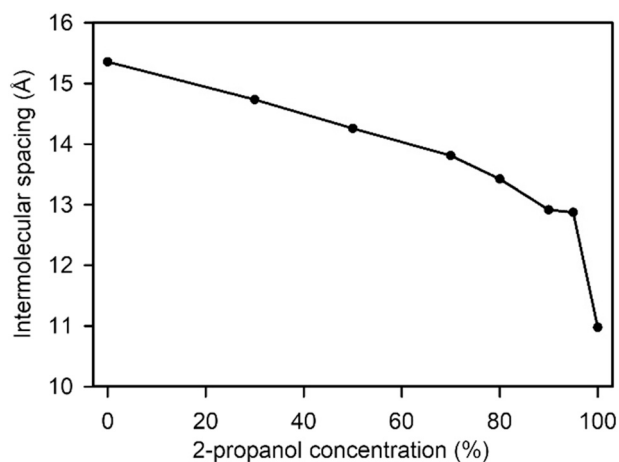


Fig. 10. Intermolecular spacing from WAXS for collagen in 0–100 % 2-propanol.

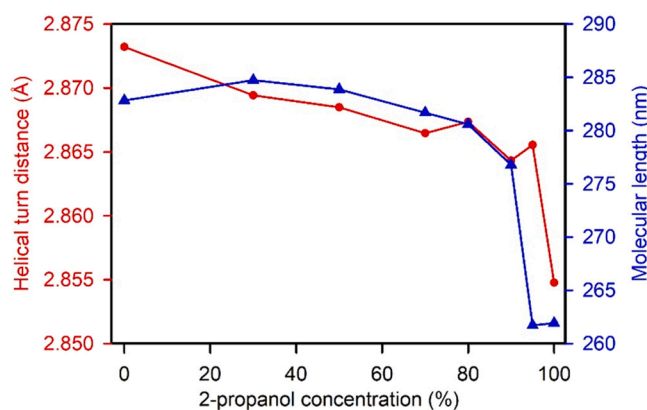


Fig. 11. Relative molecular length derived from the D-period (blue, triangles) and overlap length compared with the helical turn length (red, circles) for collagen in 0–100 % 2-propanol. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

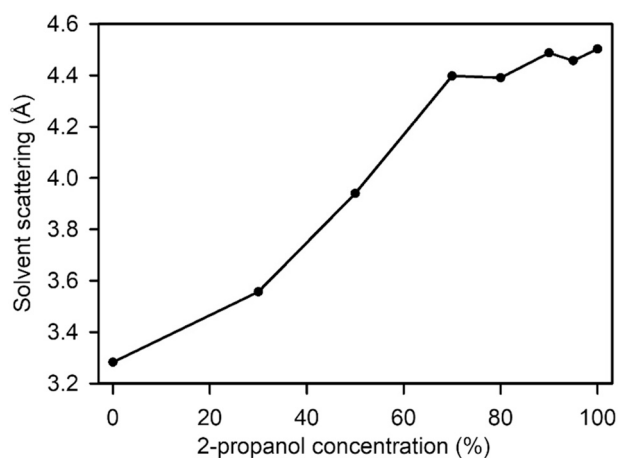


Fig. 12. Approximate position of broad scattering peak due to solvent from WAXS patterns of collagen in 0–100 % 2-propanol.

(with the overlap region decreasing), so that the length of the fibril itself does not shrink as the collagen molecules shrink. This is possible if the collagen molecules slide past each other within the fibril. Eventually the shrinkage in the collagen molecule cannot be accommodated by the

increase in the gap region and the D-period then decreases with the proportional decrease in length of the whole collagen fibril. Throughout this, the diameter of the collagen molecule is decreasing, and also, the fibril diameter decreases in line with the molecular diameter, as measured previously [7]. The shrinkage in the length of the tropocollagen is partly reflected in the change in length of the helical turn, but this only reflects a small portion of the length change.

This dehydration also results in increased stiffness, with the change in stiffness seen across the whole range of dehydration [7] suggesting that important structural changes are taking place even in the early stages of dehydration.

4.1. Collagen length changes – fibril and molecule

The overlap measured here decreases from 0.479 to 0.404 nm, a decrease of 15.7 %. The D-period decreases from 63.2 nm to 59.5 nm, a decrease of 5.9 %. The molecular length calculated from the D-period and the overlap therefore drops from 282.8 nm to 261.9 nm, a decrease of 7.4 %. The limit of molecular length shrinkage due to an increase in the gap (if the fraction gap could increase from 0.5 to 1.0) but with no change to the D-period is 11.1 %. The molecular length change based on the measured helical turn change (the helical rise per residue) is from 2.873 to 2.854 Å or 0.66 %, a rather small amount compared with the other changes measured.

But, of more interest is the way that the shrinkage occurs in the earlier stages of dehydration. From fully hydrated collagen to partial dehydration using 50 % 2-propanol the D-period increases, in other words the collagen fibril gets longer during this phase of dehydration. So how is the dehydration accommodated? It is accommodated by a decrease in the length of the overlap region of the fibril. Quantifying this, the D-period over this range of dehydration increases from 62.83 to 63.23 nm (0.64 %), while the overlap decreases from 0.479 to 0.457 Å (4.6 %) resulting in a calculated length decrease from 280.41 to 279.02 nm (0.50 %). The helical rise per residue decreases from 2.873 to 2.868 (0.17 %) over this range. So in this early stage of dehydration, the collagen fibril does not shrink in length, rather it gets slightly longer, but the shrinkage due to dehydration is accommodated entirely by a decrease in the overlap region and an increase in the gap region.

It is rather odd that the total D-period should increase slightly. However, it has been shown that D-period strain under tensile forces can slightly underestimate fibril strain at low strain (up to 0.3 %) when there is strongly twisted collagen fibrils such as in skin where the twist can be up to 17° [13]. Perhaps then, during drying, the twist could increase so that despite a slightly increasing D-period the length of the fibril is not actually increasing.

The intermolecular spacing decreases from 15.4 Å to 11.0 Å, a 29 % decrease, over the full range of dehydration measured here. From fully hydrated in water to dehydration by 50 % 2-propanol, the intermolecular spacing in collagen fibrils decreases from 15.4 Å to 14.3 Å, a 7.2 % decrease. This will have a dominant effect on the volume change because this represents shrinkage in two dimensions, whereas the length change represents just one dimension.

These changes to the collagen fibril at the two stages of dehydration is graphically illustrated in Fig. 13.

There appears to be disagreement in this data between the change in collagen molecule length (determined by the sum of 4 D-periods and an overlap) and the change in collagen molecule length measured by the helical rise per residue. One possible explanation for this, although we do not provide evidence for it here, could be slippage of the collagen molecules within the tropocollagen structure (commonly referred to as a tropocollagen molecule). The complete tropocollagen structure could perhaps shorten, with little change to each of the three collagen molecules making up this structure, by the sliding or rearrangement of the collagen molecules within the structure.

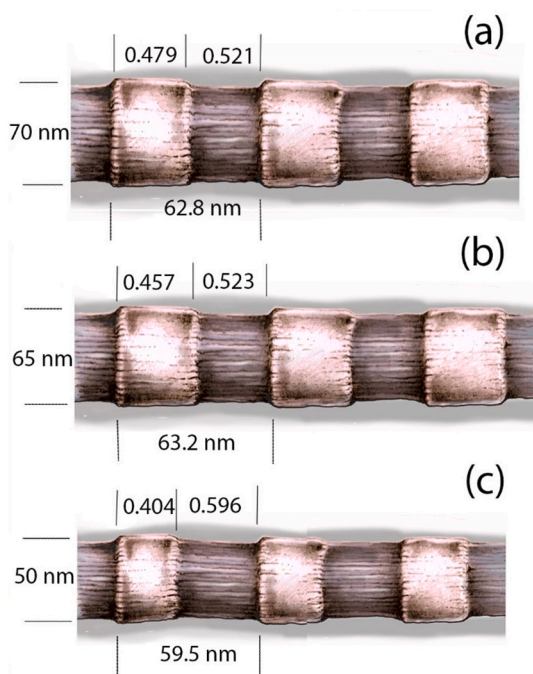


Fig. 13. Schematic of some of the changes taking place in a collagen fibril as it becomes dehydrated (not to scale): a) fully hydrated, b) partially dehydrated, c) severely dehydrated. Dimensions for diameter and D-period are in nm, and for the gap and overlap as a fraction of one D-period.

4.2. Air drying versus 2-propanol dehydration

Dehydration by alcohol of biological materials is a widely adopted practice. The experiments described here have used 2-propanol for dehydration. Alcohol has the advantage of a drying medium over removal of water by a dry atmosphere because it ensures the hydration state is more uniform throughout the material. The concentration driver for alcohol to penetrate the collagen fibril and exchange with the water is much greater than for gas phase diffusion of water out of the fibril. A further disadvantage of air drying of collagen is that during X-ray analysis the surface could dry out (or become wetter if the samples are periodically rewetted during collection of consecutive X-ray scattering patterns), whereas with the collagen sample fully saturated in a liquid solvent (2-propanol–water mixtures) the system does not change with time. This study using drying by 2-propanol should therefore also be applicable to dehydration of collagen by air drying.

4.3. Shrinkage with dehydration versus stretching under tension

With dehydration the collagen fibril shrinks. Under mechanical tension the collagen molecule stretches. There is quite a large body of work on D-period changes with tension and some on the intermolecular spacing and fibril diameter changes [5,14–18]. It is interesting to consider whether with tension the change in D-period does not reflect the full amount of force applied to the fibrils because mechanical stretching could be accommodated similarly to in shrinkage, by an increase in the gap. Modelling and atomic force microscopy measurements have shown that tropocollagen fibrils slide past each other with tension with a sawtooth force curve due to the shape of the interaction between adjacent tropocollagens [19]. The modelling suggested that molecular elongation could account for 1.7 % of the strain in the first 2 % total strain, whereas intermolecular mechanisms such as molecular slippage and gap increase account for only the remaining 0.3 % strain, which is rather different to what is reported here for dehydration of collagen.

Fibril strain is found to be smaller than tissue (applied) strain [17,20] and can depend on the amount of cross-linking [20,21].

4.4. Collagen shrinkage on a macro scale (skin drying)

How does this study of collagen dehydration at the fibrillar level and smaller length scales relate to drying of collagen tissues such as skin? It is important for skin retain a moisture level of 10–15 % to remain supple and intact [22]. When the collagen in the skin starts to dry, the length of the fibrils, and therefore perhaps the lengths of the fibres and skin, which has highly aligned collagen [23,24], do not start to shrink with initial dehydration. This is because shrinkage due to dehydration is largely taken up by an increase in the gap and decrease in overlap but no change in fibril length initially. This is an effective mechanism by which collagen tissues can accommodate changes in moisture content without major dimensional changes. However, it has been shown here that the collagen molecules do pack more closely together, and therefore the diameter of collagen fibrils decrease even at the early stages of dehydration. This is likely therefore to lead to some shrinking of tissue, especially in the direction normal to the Langer lines (lines of collagen orientation in the skin).

5. Conclusions

It has been shown that dehydration of collagen fibrils results in a two-stage dehydration process. Initially there is little change to the D-period and the dehydration results in a decrease in the overlap length and increase in the gap length to keep an approximately constant length fibril. The helical turn distance decreases, although not by enough to account for the increase in gap length. There is a decrease in the intermolecular spacing laterally between molecules. At the second stage of dehydration the D-period decreases and the overlap length decreases further leading to a significant decrease in collagen fibril length. The helical turn distance decreases further, so too does the intermolecular spacing between molecules. This mechanism explains why collagen materials are resistant to gross structural change in the direction of alignment of the collagen fibrils in the early stages of dehydration, and shows why they may then suffer from sudden external shrinkage with further dehydration.

CRediT authorship contribution statement

RGH, KHS and CKL performed the SAXS experiments, RGH analyzed the data, RGH wrote the manuscript. All the authors contributed to the overall scientific interpretation and edited the manuscript.

Data availability

Scattering data is available on request.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

Acknowledgements

This research was undertaken on the SAXS/WAXS beamline at the Australian Synchrotron, part of ANSTO, with beamtime awards M14902 and M15716. The New Zealand Synchrotron Group provided travel funding. TEI Biosciences, Waltham, USA, donated the collagen materials.

References

- [1] M.J. Turunen, H. Khayyeri, M. Guizar-Sicairos, H. Isaksson, Effects of tissue fixation and dehydration on tendon collagen nanostructure, *J. Struct. Biol.* 199 (3) (2017) 209–215.
- [2] T.J. Wess, J.P. Orgel, Changes in collagen structure: drying, dehydrothermal treatment and relation to long term deterioration, *Thermochim. Acta* 365 (1–2) (2000) 119–128.
- [3] A. Bigi, A.M. Fichera, N. Roveri, M.H.J. Koch, Structural modifications of air-dried tendon collagen on heating, *Int. J. Biol. Macromol.* 9 (3) (1987) 176–180.
- [4] P. Fratzl, N. Fratzl-Zelman, K. Klaushofer, Collagen packing and mineralization: an x-ray scattering investigation of Turkey leg tendon, *Biophys. J.* 64 (1) (1993) 260–266.
- [5] H.C. Wells, K.H. Sizeland, H.R. Kayed, N. Kirby, A. Hawley, S.T. Mudie, R. G. Haverkamp, Poisson's ratio of collagen fibrils measured by SAXS of strained bovine pericardium, *J. Appl. Phys.* 117 (4) (2015), 044701.
- [6] L.G. Gonzalez, J. Hiller, N.J. Terrill, J. Parkinson, K. Thomas, T.J. Wess, Effects of isopropanol on collagen fibrils in new parchment, *Chem. Cent. J.* 6 (1) (2012).
- [7] H.C. Wells, K.H. Sizeland, S.J.R. Kelly, N. Kirby, A. Hawley, S. Mudie, R. G. Haverkamp, Collagen fibril intermolecular spacing changes with 2-propanol: a mechanism for tissue stiffness, *ACS Biomat. Sci. Eng.* 3 (10) (2017) 2524–2532.
- [8] D. Cookson, N. Kirby, R. Knott, M. Lee, D. Schultz, Strategies for data collection and calibration with a pinhole-geometry SAXS instrument on a synchrotron beamline, *J. Synchrotron Radiat.* 13 (2006) 440–444.
- [9] H. Suhonen, M. Fernandez, R. Serimaa, P. Suortti, Simulation of small-angle x-ray scattering from collagen fibrils and comparison with experimental patterns, *Phys. Med. Biol.* 50 (22) (2005) 5401–5416.
- [10] G. Fessel, Y. Li, V. Diederich, M. Guizar-Sicairos, P. Schneider, D.R. Sell, V. M. Monnier, J.G. Snedeker, Advanced glycation end-products reduce collagen molecular sliding to affect collagen fibril damage mechanisms but not stiffness, *PLoS ONE* 9 (11) (2014).
- [11] A. Narten, H. Levy, Liquid water: molecular correlation functions from x-ray diffraction, *J. Chem. Phys.* 55 (5) (1971) 2263–2269.
- [12] T. Takamuku, K. Saisho, S. Aoki, T. Yamaguchi, Large-angle X-ray scattering investigation of the structure of 2-propanol–water mixtures, *Z. Naturforsch.* 57 (12) (2002) 982–994.
- [13] M.P. Leighton, A.D. Rutenberg, L. Kreplak, D-Band Strain Underestimates Fibril Strain for Twisted Collagen Fibrils at Low Strains, *J. Mech. Behav. Biomed.* 2021.
- [14] P. Fratzl, K. Misof, I. Zizak, G. Rapp, H. Amenitsch, S. Bernstorff, Fibril structure and mechanical properties of collagen, *J. Struct. Biol.* 122 (1997) 119–122.
- [15] H.C. Wells, K.H. Sizeland, N. Kirby, A. Hawley, S. Mudie, R.G. Haverkamp, Acellular dermal matrix collagen responds to strain by intermolecular spacing contraction with fibril extension and rearrangement, *J. Mech. Behav. Biomed.* 79 (2018) 1–8.
- [16] N. Sasaki, S. Odajima, Stress-strain curve and young's modulus of a collagen molecule as determined by the x-ray diffraction technique, *J. Biomech.* 29 (5) (1996) 655–658.
- [17] M.M. Basil-Jones, R.L. Edmonds, G.E. Norris, R.G. Haverkamp, Collagen fibril alignment and deformation during tensile strain of leather: a SAXS study, *J. Agric. Food Chem.* 60 (5) (2012) 1201–1208.
- [18] J.S. Bell, S. Hayes, C. Whitford, J. Sanchez-Weatherby, O. Shebanova, C. Vergari, C. P. Winlove, N. Terrill, T. Sorensen, A. Elsheikh, K.M. Meek, The hierarchical response of human corneal collagen to load, *Acta Biomater.* 65 (2018) 216–225.
- [19] A. Gautieri, M.I. Pate, S. Vesentini, A. Redaelli, M.J. Buehler, Hydration and distance dependence of intermolecular shearing between collagen molecules in a model microfibril, *J. Biomech.* 45 (12) (2012) 2079–2083.
- [20] R. Puxkandl, I. Zizak, O. Paris, J. Keckes, W. Tesch, S. Bernstorff, P. Purslow, P. Fratzl, Viscoelastic properties of collagen: synchrotron radiation investigations and structural model, *Philos. Trans. R. Soc.* 357 (2002) 191–197.
- [21] H.R. Kayed, K.H. Sizeland, N. Kirby, A. Hawley, S.T. Mudie, R.G. Haverkamp, Collagen cross linking and fibril alignment in pericardium, *RSC Adv.* 5 (2015) 3611–3618.
- [22] A. Pons-Guiraud, Dry skin in dermatology: a complex physiopathology, *J. Eur. Acad. Dermatol. Venereol.* 21 (2007) 1–4.
- [23] H.C. Wells, R.L. Edmonds, N. Kirby, A. Hawley, S.T. Mudie, R.G. Haverkamp, Collagen fibril diameter and leather strength, *J. Agric. Food Chem.* 61 (47) (2013) 11524–11531.
- [24] P.D. Verhaegen, H.J. Schouten, W. Tigchelaar-Gutter, J. van Marle, C.J. van Noorden, E. Middelkoop, P.P. van Zuijlen, Adaptation of the dermal collagen structure of human skin and scar tissue in response to stretch: an experimental study, *Wound Repair Regen.* 20 (5) (2012) 658–666.

Collagen dehydration

Haverkamp RG

2022-09-01

14/03/2024 - Downloaded from MASSEY RESEARCH ONLINE