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Fracture behaviour of cross-linked collagen fibres

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Mechanical properties such as stress - strain, fracture behaviour and thermal behaviour were studied for cross-linked collagen fibres. The shrinkage temperature of cross-linked fibres shows increase in temperature when compared to the control. The results on measurements of breaking strength and strain show significant change when compared with that of the control. The morphological features of the fractured ends of cross-linked fibres were indicative of certain specific patterns. A critical observation of these patterns indicate the role played by the nature of cross-linking agents on the mechanism of failure of these fibres.

1. Introduction

Collagen fibres, are the major constituents of skin, tendon and ligaments. The mechanical properties of collagen fibres have been a topic of great interest [1–4]. Morgan [4] reported that there is an optimum rate of loading at which the collagen fibres give maximum strength and extension. The stress - strain behaviour of native and dry collagen fibres have been reported in the literature [5, 6]. In these experiments, rat tail tendon (RTT), a pure form of collagen with parallel orientation of the fibrils, has proved to be an excellent choice [5].

Cross-links in collagen can be either intra molecular or intermolecular. In either case, a principal function of cross-links is the stabilization of an organized structure. One of the commonly used strategies for the stabilization of collagenous matrices is to introduce additional (artificial) cross-links. Chemical reagents in particular, bifunctional aldehydes like glutaraldehyde, have been found to, participate in the formation of new cross-links. The use of formaldehyde to introduce new cross-links is well known. In an industrial application of collagen in the form of leather, chromium salts are extensively used. The nature of cross links [7–11] are as follows : Chrome tanning involves complexation of chromium with the carboxyl groups in the side chains of asparatic acid and glumatic acid residues of collagen molecules. Formaldehyde tannage results in short methylene bridges which are covalent in character where as in glutaraldehyde it is due to cross-links by lysine or hydroxylysine residues. It has been reported that cross-linking, hydrogen bonding, temperature and solvents influence the viscoelastic behaviour of collagen [12, 13]. Fracture behaviour of collagen fibres has been the subject of interest in the recent past [14, 15]. The aim of this present investigation is to study the mode of fracture as influenced by the nature of cross-linking of collagen fibres.

2. Materials and methods

Collagen fibres were obtained from the tail of male albino rats (6 months old) after sacrificing them. The tails were cut and the skin peeled off, before teasing out with the tendons with least force from the tails [5]. The fibres were washed well in distilled water and divided into three sets. One set was treated with 1% basic chromium sulphate (BCS) solution at pH 3.8. Another set of fibres were treated with formaldehyde (1% solution) and the third set were treated with glutaraldehyde (1% solution) at pH 8. Before testing the samples they were equilibrated in the respective solutions for 24 hrs. The fibres were tested in the Instron Universal Testing machine (model 1112), at an extension rate of 5 mm/min. The gauge length was fixed as 10 mm.

Small strips of fibres were cut and placed on microscopic slides mounted on a holder carrying a thermometer with heating facility at the rate of 2°C/min The shrinkage temperature data were collected using standard methods [16].

The fractured ends were mounted on aluminum stubs using silver dag and coated with gold in Edwards (SC 500) sputter coater. The samples were scanned in Stereoscan 440, Scanning Electron Microscope.

3. Results and discussion

The tensile strength of the fibres decreases after tanning (Fig. 1) in conformity with previous workers [5, 17]. The formaldehyde-tanned fibres were found to break at the lowest value of 82.34 MPa, glutaraldehyde at 108.43 MPa and BCS tanned fibres at 105.66 MPa. For the control fibres (dry) the breaking stress is 120.02 MPa. Fig. 1 reveals that the tanned fibres yield more when tensile forces are applied i.e., for a given stress the strain is more for those fibres when compared to control fibres. Considering the strain at break, it is

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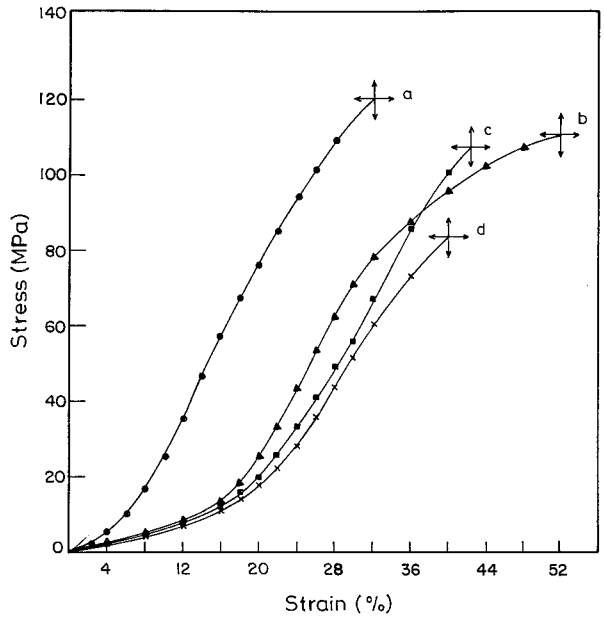

Figure 1 Stress - Strain behaviour of (a) RTT and RTT cross-linked with (b) BCS (c) glutaraldehyde and (d) formaldehyde.

lowest for formaldehyde 40%, with glutaraldehyde at 42% and for BCS tanned fibres it is 47%. These values are greater than the control, which breaks at 31%. The BCS tanned fibres are highly crimped when compared to the fibres tanned in other ways [17]. Hence its extension and tensile strength is higher than that of the fibres tanned with formaldehyde and glutaraldehyde. Unipoint and multipoint cross linking of collagen by chromium occurs leading to the increased stabilized structure, which would help in the uniform distribution of the stresses resulting in the higher breaking strain [18].

In the case of formaldehyde tanned fibres the cross-links formed is less and also it is weaker than the glutaraldehyde fibres. Hence the breaking stress and the strain are less than that of glutaraldehyde and chrome tanned fibres as observed in the shrinkage temperature (Table I). The collagen fibres tanned with BCS have higher shrinkage temperature when compared to aldehyde tanned fibres. It has been suggested that T_S depends on the size of the co-operating units in the shrinking process, the larger the unit, slower is the kinetics, the higher the shrinkage temperature, hence the observed result [19, 20].

TABLE I Shrinkage temperature of RTT and RTT crosslinked with (BCS), glutaraldehyde and formaldehyde

Sample	T_s(°C)
Control	63 ± 1
BCS	107 ± 1
Glutaraldehyde	92 ± 1
Formaldehyde	87 ± 1

An extensive study carried out on the mode of fracture of fibres using scanning electron microscopy has led to the classification of the mode of fracture into three principal patterns. They are : (i) smooth fracture, fracture occurring in a single plane perpendicular to the fibre axis; (ii) step fracture, with fracture initiating in a plane perpendicular to the fibre axis and propagating along the fibre axis, resulting in splitting of the fibre along its axis; (iii) fibrillation, with the fractured end split open into the smaller fibrils [21].

The fractured end of collagen fibres is given in Fig. 2. During the application of the load (the fibres being tested at 5 mm/min) there is sufficient time for the fibrils to orient themselves along the direction of applied force. It can be seen from the Fig. 2 that the fracture is similar to the step fracture and the splitting of the fibres can be at the tip the fibre axis. [21]. Occasionally, fibrils act like flaws in the fibres at which fracture can initiate. The step fracture results from stress concentrations at the cracks, which are propagated in between the hierarchical planes along the fibre axis. The simultaneous propagation of more than one crack along the fibre axis, can result in splitting of the fibre before breaking. This behaviour is different from that in the elastoidin fibres, which is similar to collagen [22].

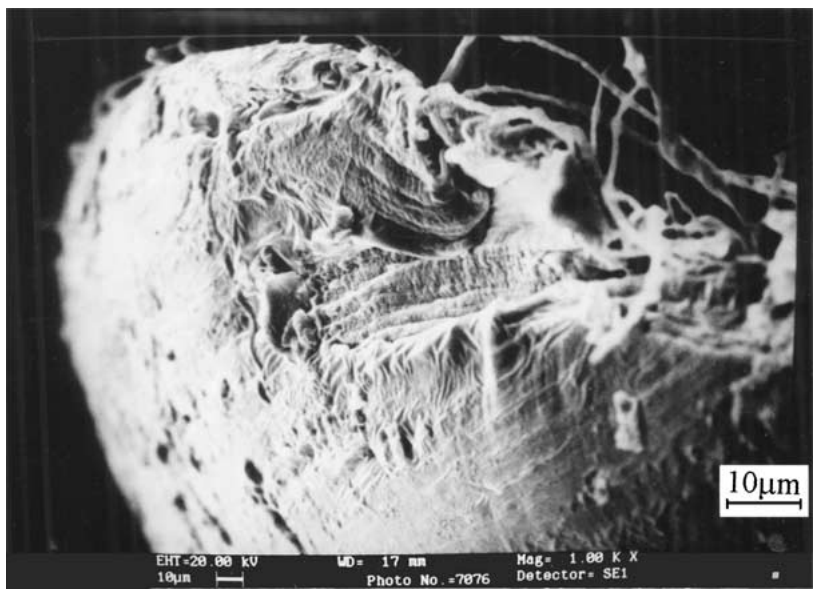

Figure 2 Scanning electron micrograph of RTT.

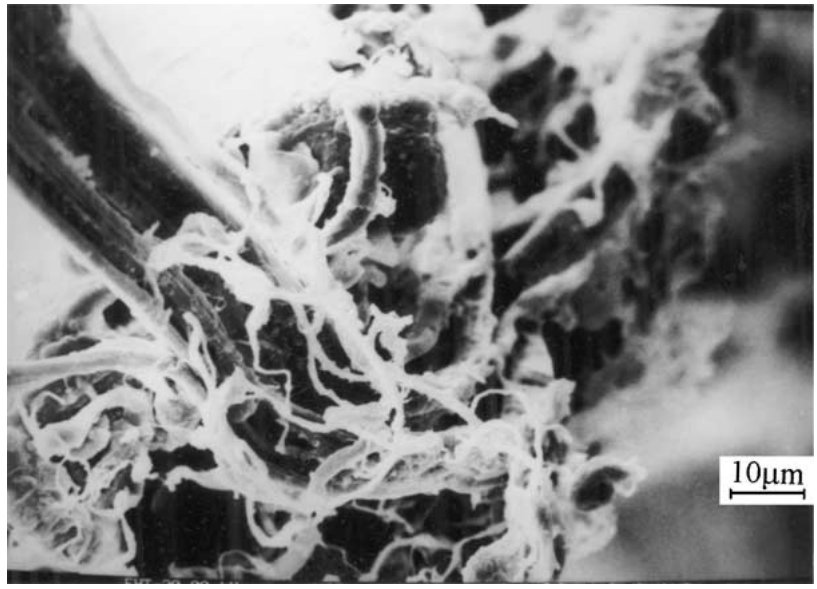

Figure 3 Scanning electron micrograph of RTT cross-linked with BCS.

In Fig. 3 the fractured ends of BCS cross-linked fibres is given, in which the fibrillation effect (the fractured end is split open into smaller fibrils and has the appearance of the bristles of a paint brush) is seen. Moreover, the degree of splitting is high (number of fibrils are more when compared to native) and the fibre morphology is in between step and fibrillation. The fibre splits into smaller fibril bundles. Cross-linking of collagen with BCS leads to considerable increase in the shrinkage temperature. Both thick and thin fibril bundles are seen similar to dry RTT fibers [15]. The size of the bundle varies unevenly. However, there is one similarity, all the fibrils (thick and thin) seem to curl at the broken end and the tip of the bundle shows cohesion. The overall effect is to pull out the fibrils from the fibre.

In glutaraldehyde treated RTT the sheath like structure could be seen at the broken end. We can see relatively large crevices at the fractured ends (Fig. 4). This might also have been initiated from the surface of the fibre, which suggests that fracture propagation occurs by secondary cracks generated as a result of stress concentration building up at the periphery of the primary crack [23]. The application of load being slow, results in fracture occurring smoothly. There is a row of sheath like fibril bundles coming out of the fibre. This may be the last one to hold the entire load and hence the sheaths are pulled out of the fibre as seen in the broken end.

The cross-linking of collagen with formaldehyde leads to smooth and brittle fracture. There are no fibrils, which could be seen at the fracture edge. From the Fig. 5 we observe that the crack might have been initiated in diametrically opposite directions (left side and right side). A hole on the right side and another on the left side. The crack might have been originated at both the right hand side and left hand side and smooth fracture could be seen upto the middle of the fractured end. Both of them have propagated upto the middle and

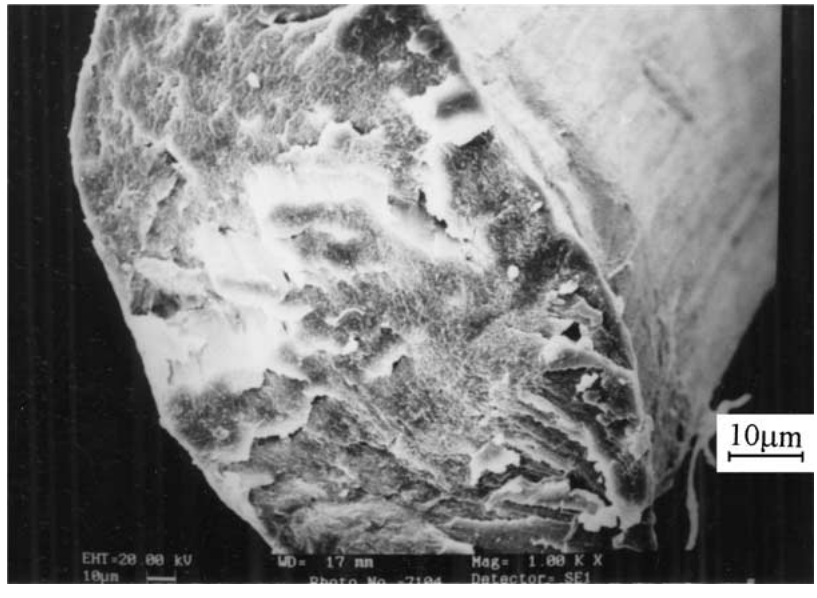

Figure 4 Scanning electron micrograph of RTT cross-linked with glutaraldehyde.

Figure 5 Scanning electron micrograph of RTT cross-linked with formaldehyde.

where sudden catastrophic failure might have occurred. This could be seen by scale like structure at the centre.

This study shows that the fracture behaviour of control (dry RTT), BCS treated RTT, glutaraldehyde and formaldehyde treated RTT are different. The shrinkage temperature dose not directly reflect the strength of the RTT or the pattern of fracture in RTT.

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