

***Positron annihilation lifetime spectroscopy of
mechanically milled protein fibre powders and their
free volume aspects***

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Positron annihilation lifetime spectroscopy of mechanically milled protein fibre powders and their free volume aspects

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Abstract. The present study reports the fabrication of ultra-fine powders from animal protein fibres such as cashmere guard hair, merino wool and eri silk along with their free volume aspects. The respectively mechanically cleaned, scoured and degummed cashmere guard hair, wool and silk fibres were converted into dry powders by a process sequence: Chopping, Attritor Milling, and Spray Drying. The fabricated protein fibre powders were characterised by scanning electron microscope, particle size distribution and positron annihilation lifetime spectroscopy (PALS). The PALS results indicated that the average free volume size in protein fibres increased on their wet mechanical milling with a decrease in the corresponding intensities leading to a resultant decrease in their fractional free volumes.

1. Introduction

Natural fibres derived from animal sources are proteinaceous in nature. Their molecules consist of a long polypeptide chain constructed from the eighteen commonly encountered amino acids. Due to the diverse chemical nature of these amino acids, different nucleophilic groups are available on these fibres such as amino (-NH₂), hydroxyl (-OH) or thiol (-SH) groups which are present in the amino side-chains of the polypeptide [1]. These functional groups can be used as active sites for binding of molecules targeting specific applications [2]. To explore the functional properties of animal protein fibres, there has been growing interest in converting protein fibres such as cashmere guard hair, wool and silk into fine powders [3,4]. Apart from the various possible applications [5], animal protein fibre

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powders have demonstrated to improve absorption kinetics towards organic [6] and inorganic [7] molecules due to their increased specific surface area and reactivity.

The absorption kinetics is mainly governed by the free volumes, also known as cavities, voids, holes and pores, present in the sorbent, besides its specific surface area and reactivity. These free volumes can act as a transport system for the molecular diffusion into the bulk of the sorbent material. Therefore, the mean free volume size, its distribution and the fractional free volume (i.e. total free volume as a percentage of the complete volume of the material) in the sorbent are important factors which determine the absorption capacity of the sorbent for the given molecules [8]. Due to the highly realized potential of animal protein fibre powders in the space of molecular absorption, this study reports on the free volume aspects of certain animal protein fibres such as cashmere guard hair, merino wool and eri silk fibre and the effect of their mechanical milling on the same using positron lifetime spectroscopy (PALS).

2. Experimental

Cashmere guard hair (CGH) supplied by *M/s Cashmere Connections Pty Limited, Australia*, scoured merino wool fibres (MWF) procured from *M/s Australian Country Spinners, Australia* and eri silk (ES) in the form of cocoons from north east India were used for their respective CGHP, MWFP and ESP powder preparations. MWF were used as received, while CGH and ES cocoons were cleaned prior to milling. CGH fibres were cleaned on a *Mesdan* sample carding machine with three passes, followed by manual removal of any adhering traces of foreign matters. Whereas, ES cocoons were degummed for 20 min in a *Thies* laboratory dyeing machine using laboratory grade 2 g/L Na₂CO₃ and 0.6 g/L sodium dodecyl sulphate at 100 °C with a material-to-liquor ratio of 1:25 (kg/L). The cocoons were subsequently washed thoroughly with warm distilled water followed by copious amounts of cold distilled water. The degummed ES cocoons were finally dried at 60 °C overnight. The cleaned CGH, merino wool fibres and degummed ES cocoons were converted into dry powders by following the previously developed powder fabrication sequence in our research group [3].

The morphologies of the milled powder samples and corresponding fibres were observed under a Scanning Electron Microscope, SEM (*Zeiss Supra 55VP*) at 5-10 kV accelerated voltage and 8-9 mm working distance. The samples were coated with 15 nm gold layer on a sputter coater (*Bal-Tec sputter Coater SCD 050*) before imaging. All powder samples' particle size distribution was measured by using the laser diffraction technique on *Mastersizer 2000* from *Malvern Instruments, UK*. The powder particles were dispersed in *propan-2-ol* (from *Sigma-Aldrich*) during the measurement. The particle size measurement was repeated three times and the volume based particle size distribution was reported along with the mean particle size (d(0.5)). As there was no significant variation among the measurements, no error bars are plotted while reporting the results. For each of the studied protein fibre types and their respective powder samples, PALS measurements were performed at room temperature. The lifetime spectra of positrons were measured using a conventional lifetime system with digital oscilloscope (*LeCroy Wavepro*). In order to determine a number of lifetime components, deconvolution was performed on the lifetime spectra. The observed spectra were analysed with a time resolution of about 170 ps by using the *RESOLUTION* computer program [9]. The free volume radius (R) was calculated as per the simple relation (equation 1), according to Nakanishi et al [10].

$$\tau_3 = \frac{1}{2} \left[1 - \frac{R}{R_0} + \frac{1}{2\pi} \sin\left(\frac{2\pi R}{R_0}\right) \right]^{-1} \quad (1)$$

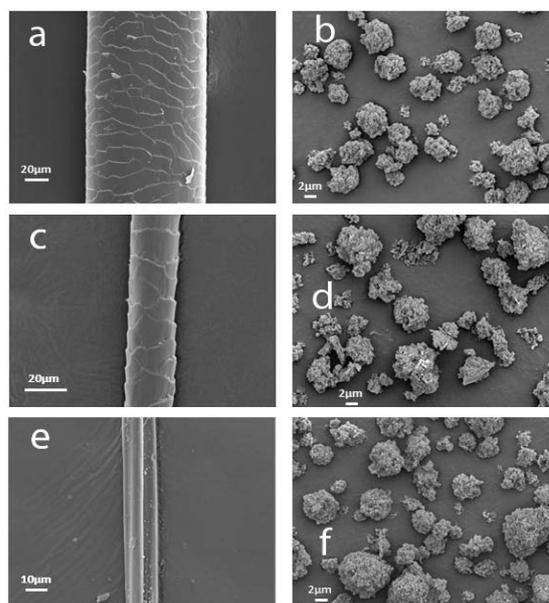


Figure 1. SEM image of a. CGH, b. CGHP, c. MWF, d. MWFP, e. ES and f. ESP

where, τ_3 (o-Ps lifetime) and R (free volume radius) are expressed in ns and nm, respectively and $R_0 = R + \Delta R$, where ΔR is a constant for an electron layer thickness (0.166 nm). The average free volume size (V_f) was then simply evaluated as $V_f = (4/3)\pi R^3$, whereas the fractional free volume (F_v) was estimated as $F_v = CV_f I_3$ [11] and C may be assigned a value of 1 due to absence of variation of free volume data with temperature.

3. Results and Discussion

The surface morphology of CGH fibre is very much similar to MWF with near cylindrical shape and surface scales pointing towards the fibre tip (figure 1a and 1c), whereas ES fibres have well known smooth and trilobal morphology (figure 1e). CGH fibres are distinctly large in diameter, $\sim 80 \mu\text{m}$ as compared with MWF and ES fibres which were typically around $20 \mu\text{m}$. Although each protein fibre type had distinct surface morphology and structure, the spray dried particles from their 6 hr attritor milled slurry appeared very similar in shape and size. The particles were nonspherical, globular in shape with a mushroom like structure (figure 1b, 1d and 1f). The volume based mean particle sizes ($d(0.5)$) were $4.63 \mu\text{m}$, $6.05 \mu\text{m}$ and $5.85 \mu\text{m}$ for CGHP, MWFP and ESP respectively (figure 2).

Figure 3 shows PAL spectra of CGH. The spectra were similar for all fibre and powder samples. We observed that three lifetime components give the best χ^2 and reasonable standard deviation. PAL component, τ_1 , is the lifetime of para-positrons (p-Ps) and τ_2 is related to free annihilations. The longest lifetime component (τ_3) is attributed to the pick-off annihilation of ortho-positrons (o-Ps) in the free volume sites present in the amorphous regions of the sample [11]. Thus, the τ_3 component of the PAL spectra and its corresponding intensity (I_3) keeps relevance from the molecular diffusion point of view.

Even though the PAL spectra were similar for all the studied fibre and powder samples (figure 3), slight differences in o-Ps (τ_3) and their corresponding intensities (I_3) were observed (table 1). From the o-Ps results, mean free volume radius (R), mean free volume size (V_f) and fractional free volume (F_v) were calculated and listed in table 1. Among the studied animal protein fibre samples, ES has the highest F_v (17.9 %), whereas the CGH and MFW have similar F_v (~ 15 %). Although relatively small, but very similar values for silk, wool and few other hair fibres have also been reported elsewhere [12]. Most importantly, table 1 also depicts that the F_v for CGH, MWF and ES has decreased on milling by 3.56 %, 19.68% and 11.61 % respectively.

On milling, the intensity of o-Ps lifetime component τ_3 was decreased for all the animal protein fibre powders (table 1). It is likely that the wet conditions during the attritor milling of the protein fibres significantly decreased their glass transition temperature [13] and made their respective molecules in the amorphous region more labile at the milling temperature. Under the influence of compressive forces of milling media on the labile protein fibre molecules, the free volumes in the amorphous region would have undergone coalescence phenomenon, resulting in an increase in the mean free volume size and a decrease in its intensity. From the molecular absorption application point

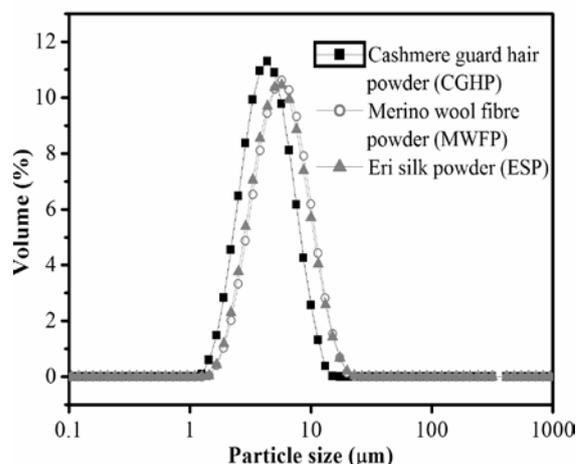


Figure 2. Particle size distributions of mechanically milled protein fibre powders

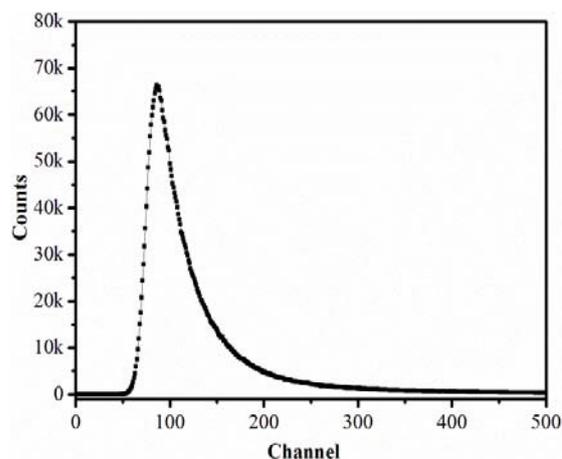


Figure 3. PAL spectra of cashmere guard hair (CGH)

of view, although the ultrafine protein fibre powder particles would provide improved surface area for immediate molecular adsorption, the decreased F_v is likely to retard the time dependant molecular diffusion phenomenon. Similar results have been realized by us [12] where Zn^{2+} and Cr^{6+} have been instantaneously adsorbed onto the CGHP with negligible diffusion controlled, time dependent absorption of the species.

Table 1. Analysed PAL spectra data for animal protein fibres and their mechanically milled powder

Sample		o-Ps	Intensity	Average free	Average free	Fractional free
		Lifetime (ns)	(%)	volume radius (Å)	volume size (Å ³)	Volume
		τ_3	I_3	R	V_f	F_v
Cashmere guard hair	Fibre	1.79	19.5	2.65	77.8	15.17
	Powder	1.79	18.6	2.66	78.7	14.63
Merino wool	Fibre	1.81	19.3	2.67	79.5	15.34
	Powder	2.00	12.6	2.86	97.8	12.32
Eri silk	Fibre	1.85	21.3	2.72	84.1	17.91
	Powder	1.81	19.7	2.68	80.4	15.83

Thus, protein fibre powders fabricated by the present wet attritor milling route are likely to be more suitable in applications where ultrafine materials, accompanied with biocompatibility and biodegradability, are required. However, molecular absorption trajectory of the potential protein fibre powder application could be more efficiently explored when the free volume aspects of the parent protein fibres get retained or improvised during their fabrication.

4. Conclusion

The wet mechanical milling of viscoelastic protein fibres promote coalescences of the free volumes present in the fibre structure, leading to the increased average free volume size, along with decrease in the corresponding relevant intensity. Consequently, the fractional free volume in the protein fibre powder gets decreased and is likely to affect their performance in molecular absorption applications. For better molecular absorption capacities of the mechanically milled protein fibre powders, free volume aspects of the parent protein fibres need to be either retained or improved by exploring different powder fabrication process parameters or routes.

Acknowledgement

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