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Dissolution Properties of Silk Cocoon Shells and Degummed Fibers from African Wild Silkmoths

[1]K.T. Addis and [2]S.K. Raina
[1]Haramaya University, P.O. Box 138, Dire Dawa, Ethiopia
[2]Commercial Insects Program, ICIPE, African Insects Science for Food and Health, P.O. Box 30772-00100, Nairobi, Kenya

Abstract: Silk cocoon shells and degummed fibers from four African wild silkmoth species were studied and compared with the industrial standard, *Bombyx mori*, for their dissolution properties. Nine M aqueous Lithium bromide, Calcium chloride and Sodium thiocyanate solution systems were used. Efficiency of the solvent systems was determined by the percentage of dissolved silk cocoon shells and degummed fibers after three hours of treatment. Degummed fibers were more readily soluble than the cocoon shells. *B. mori* cocoon shells (51.5%) and fibers (59.3%) had higher solubility than their wild counterparts. Among the wild species, *Gonometa postica* cocoon shells and degummed fibers had the highest solubility (37.3 and 51.7%, respectively). Lithium bromide was the most effective dissolving agent for both the cocoon shells and fibers (41.2 and 84.5%, respectively). *Argema mimosae*, *Anaphe panda* and *Epiphora bauhiniae* showed lower solubility across the solution systems used. The Scanning Electron micrographs showed *A. panda* fibers exhibited gelling property after dissolution while *E. bauhiniae* and *A. mimosae* had cracked and broken fibers exposing the fibriliar structures. The difference in the chemical orientation and composition of the fibers might have contributed to the variability in the dissolution behaviour.

Key words: Solubility, SEM, wild silkmoth, fibroin, silk

INTRODUCTION

Cocoon shells of sericigenuos insects are complex materials fashioned mainly by essentially two major proteins, fibroin (the core structural protein) and sericin (the surrounding glue gum). Sericin which covers the periphery of the raw silk fibers, is highly soluble in hot water and can easily be removed in the process of degumming (Nuanchai *et al*., 2010; Prasad *et al*., 2012). It is a mixture of proteins with a large number of amino acids containing hydroxyl groups and majorly contains serine (32%) and large proportion of several polar amino acids that confers it with high hydrophilicity and adhesion (Padamawar and Pawar, 2004). Fibroin, on the other hand, is not soluble in water and roughly 76% of its amino acids have non-polar side chains, the main ones among these being glycine (42-46%), alanine (30-32%) and serine (12-14%) (Zhou *et al*., 2000; Arami *et al*., 2007). The solution behaviour of Silk Fibroin (SF) is of interest due to its novel self-assembly and processing related to fiber spinning in spiders and silkworms, resulting in remarkable mechanical properties (Matsumoto *et al*., 2008; Prasong *et al*., 2010). Natural polymers, such as silks, have been gaining wide use in variety of applications besides their traditional use as a textile raw material (Teshome *et al*., 2011; Mondal *et al*., 2007; Wilaiwan *et al*., 2010). In recent years, fibroin from the domesticated silkworm *Bombyx mori* has been the dominant source for silk based biomaterials and its dissolution properties are often required for such non-textile applications. This is mainly due to the difficulty to dissolve silk fibroin in common solvents for obtaining a true solution because of its high molecular weight and crystallinity (Ki *et al*., 2007). The insolubility of silk fibroin in common organic solvents is due to the intermolecular hydrogen bonding existing in the polymer and its hydrophobic nature (Furuhata *et al*., 1994; Jin and Kaplan, 2003). Further, the fibroin dissolution is affected by the non-uniformity of fibroin in the chemical composition, the supra-molecular structure and the morphological futures of natural fibers (Sashina *et al*., 2006). The environmental factors and the processing conditions used by the silk producing insects also influence the solution behaviour and ultimately the material properties of these fibers (Matsumoto *et al*., 2008).

Despite these difficulties, the solubility of silk in certain solvents has been studied and several proper solvent systems have been successfully used to dissolve

Corresponding Author: K.T. Addis, Haramaya University, P.O. Box 138, Dire Dawa, Ethiopia

silk fibroin including Lithium bromide (Alessandrino *et al*., 2008), N-methylmorpholine-N-oxide (NMMO) hydrate (Plaza *et al*., 2008), Phosphoric acid/formic acid mixture (Ki *et al*., 2007), Calcium nitrate (Kweon *et al*., 2001; Prasong *et al*., 2010) and Calcium chloride (Miyaguchi and Hu, 2005). Ajisawa (1998) also recommended chaotropic reagents such as Lithium thiocyanate (LiSCN), LiBr, Sodium thiocynate (NaSCN) and $CaCl_2$ as dissolution reagents for SF. However, the solubility of different silk fibers in these solvent systems was reported to be inconsistent. Srihanam (2009) and Srisuwan and Srihanam (2009) found *B. mori* silk is more soluble than Eri silk. *Antherea pernyi* silk fibroin is difficult to dissolve due to the strong inter and intra-molecular interactions between fibroin molecules and chains (Kweon *et al*., 2000, 2001). In this regard, the wild silk from genus *Antheraea* is mostly studied (Dash *et al*., 2007). The African wild silkworms which produce commercially important silk are found widely distributed in different geographical regions of Africa. However, little information is available so far on their structure and properties, of which dissolution properties are no exceptions. Hence, this study reported the dissolution characteristics of four African wild silk cocoon shells and degummed fibers in three solvent systems.

MATERIALS AND METHODS

Determination of solubility of cocoon shells and degummed fibers: Cocoon shells were cut with a surgical blade and cleaned by removing the debris and other foreign materials. Twenty gram of cleaned cocoons were enclosed in wire mesh cages with a volume of 717 cm^3 and boiled with 5 g L^{-1} of Na_2CO_3 solution for 3 h (*A. mimosae* and *E. bauhiniae*), 5 h (*A. panda*), 1.5 h (*G. postica*) and 1 h (*B. mori*). Boiled cocoons were then soaked in star soft solution of 50 mL L^{-1} of distilled water for 3 min and washed with hot and cold distilled water twice. Degummed fibers were dried in oven at 110°C for 24 h and stored in desiccators prior to use. Analytical grades of Calcium chloride ($CaCl_2$), Sodium thiocyanate (NaSCN) and Lithium bromide (LiBr) were used. Nine molar aqueous solutions were prepared by dissolving 299.68, 234.48 and 218.84 g of $CaCl_2$, LiBr and NaSCN, respectively and stirring with a magnetic stirrer at room temperature for 10 min. Almost 0.1 g of air dried degummed fibers was dissolved in 10 mL of each solvent solution at 70°C for 3 h with gentle shaking of flasks at 40 rpm in an incubator (Sah and Pramamik, 2010). One gram of oven dried cocoon shell discs were also dissolved the same way. The experiment was replicated four times. The resulting solutions were allowed to settle overnight. The undissolved silk was filtered through non woven fabrics, washed, dried in a vacuum drying oven at 110°C for 24 h and weighed. The dry weight remaining was expressed as a percentage relative to the initial weight and solubility was calculated as:

$$\text{Solubility (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

where, W_1 is initial weight of the sample and W_2 is weight of the residue (Rastogi *et al*., 2001; Kweon *et al*., 2001).

Scanning electron microscopy (SEM): Dried fibroin supernatant remaining were mounted onto copper stubs using double side sticking tape and sputter-coated with gold for three minutes. The samples were then observed with SEM (Jeol Neoscope, JCM-5000 (Nikon, UK)) under an accelerating voltage of 10 kv with a beam current of 0.1 nA.

Statistical analysis: The percentage data was log transformed to stabilize variance and data were subjected to two-way Analysis of Variance (ANOVA) using General Linear Model Procedure (PROC GLM). Least Significance Difference (LSD) test was used to separate means (SAS, 2010).

RESULTS

Table 1 showed the percentage solubility of cocoon shells dissolved with 9M aqueous solutions of $CaCl_2$, LiBr and NaSCN. The results confirmed that dissolution property of cocoon shells depends on the origin of cocoon shells and the solvents used. The interaction effects of solvents and species was highly significant. *B. mori* had the highest percent solubility across all the solvents used (51.5%) and LiBr had significantly higher dissolving ability (41.2%). There was no significant difference in the solubility of cocoon shells in $CaCl_2$ and NaSCN. However, *A. mimosae* and *E. bauhiniae* showed slightly higher solubility percentage in $CaCl_2$ than NaSCN (Table 1). Among the wild silk cocoon shells, *G. postica* had higher solubility.

Table 1: Mean±SE percentage solubility of cocoon shells of four African wild silkmoths and *Bombyx mori*

	$CaCl_2$	LiBr	NaSCN	Mean
Bombyx mori	29.8±0.7^a	97.7±0.5^a	26.8±0.8^a	51.5±3.6^a
Anaphe panda	11.2±0.4^c	21.5±0.7^c	11.2±0.5^c	14.6±1.4^c
Argema mimosae	5.7±0.6^d	7.7±0.5^d	3.4±0.7^d	5.6±0.8^c
Epiphora bauhiniae	3.1±0.5^e	3.2±0.5^e	2.4±0.5^d	3.1±0.4^e
Gonometa postica	15.4±0.7^b	74.7±0.5^b	21.6±0.7^b	37.3±3.3^b
Mean	13.1±1.4^b	41.2±2.9^a	13.0±1.5^b	22.4±2.3

Means followed by the same letter in the same column are not statistically significant (p>0.001) according to least significant difference (LSD) test

The dissolution of degummed fibers also showed significant differences in interaction of species and solvents. Like the cocoon shells, *B. mori* fibers also showed highest solubility in all the solution systems used (59.3%) and LiBr proved the best solvent agent for the fibers (84.5%) (Table 2). However, unlike the cocoon shells there was significant difference in dissolution percentage between $CaCl_2$ and NaSCN (18.2 and 25.4%, respectively). *A. mimosae* and *E. bauhiniae* showed lower solubility in NaSCN than $CaCl_2$ while the other fibers dissolved more readily in the later. *G. postica* fibers were dissolved completely in LiBr solutions and the supernatant was composed of only the calcium oxalate crystals and remnants of the cocoon spines and hairs.

Much of the solid fibers of *A. mimosae*, *A. panda* and *E. bauhiniae* were observed after the 3 h of treatment which confirm that dissolution of fibers was not complete in these species. The SEM micrographs for the fibers after treatment with LiBr showed differences in the mechanism of dissolution (Fig. 1). *E. bauhiniae* fibers were cracked and signs of disintegration and fractures were observed on the surface (Fig. 1a) while fibers of *A. mimosae* were

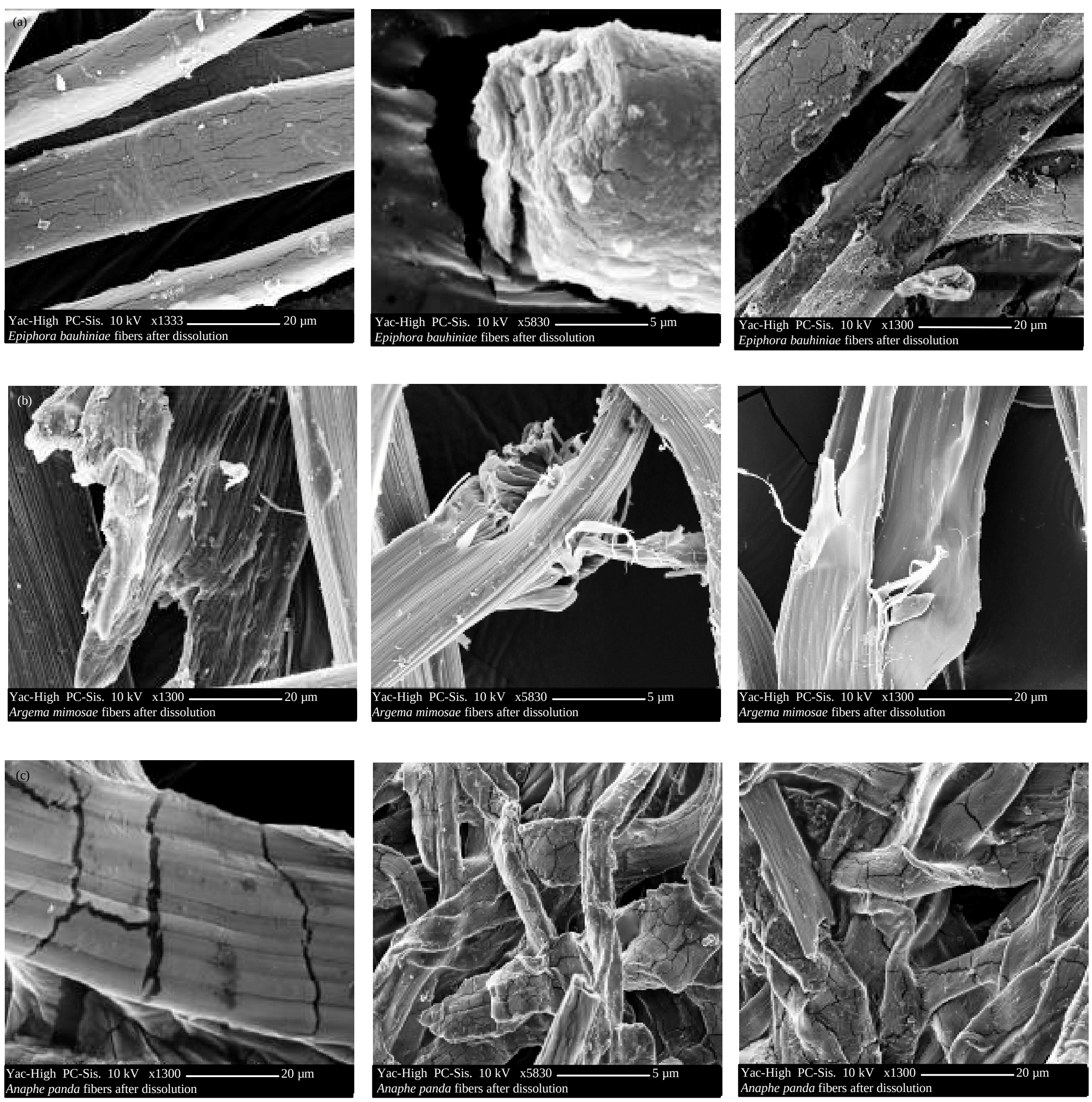

Fig. 1(a-c): Fibers of (a) *Anaphe panda*, (b) *Epiphora bauhiniae* and (c) *Argema mimosae*, after treatment with 9 M LiBr

Table 2: Mean±SE percentage solubility of degummed fibers of four African wild silkmoths and *Bombyx mori*

	$CaCl_2$	LiBr	NaSCN	Mean
Bombyx mori	29.9 ± 0.8^a	99.1 ± 0.3^a	48.8 ± 1.1^a	59.3 ± 3.4^a
Anaphe panda	12.5 ± 0.8^c	84.4 ± 0.6^b	20.5 ± 1.0^c	39.1 ± 3.6^c
Argema mimosae	12.1 ± 0.8^c	77.2 ± 0.6^c	11.2 ± 0.9^d	33.5 ± 3.5^d
Epiphora bauhiniae	14.1 ± 0.8^c	64.4 ± 0.7^d	11.5 ± 0.9^d	30.1 ± 3.1^e
Gonometa postica	22.2 ± 0.6^b	97.5 ± 0.4^a	35.4 ± 0.9^b	51.7 ± 3.6^b
Mean	18.2 ± 1.2^c	84.5 ± 1.7^a	25.4 ± 1.8^b	42.7 ± 0.0

Means followed by the same letter in the same column are not statistically significant ($p>0.001$) according to least significant difference (LSD) test

broken in to pieces exposing the individual fibrils (Fig. 1b). The fibers of *A. panda* in LiBr, however, showed a strong tendency of gelling and were difficult to handle (Fig. 1c).

DISCUSSION

The study clearly showed the African wild silk cocoon shells and degummed fibers have variability in their solubility in different solvent systems. LiBr exhibited higher dissolving ability than the other solvents. However, this is in contrast with (Srihanam, 2009; Srisuwan and Srihanam, 2009) who reported that 9 M LiBr had the least ability to dissolve *Philosamia ricini* silk fibroin. The low solubility of *A. panda*, *E. bauhiniae* and *A. mimosae* cocoon shells and degummed fibers in all the solvent systems could be due to too short time of treatment or the solvent systems were not strong enough to produce the desired level of dissolution. The temperature could also be a factor for low solubility of the cocoon shells. Other wild silks, such as *A. pernyi* silk fibroin also require a high concentration of chaotropic salts, high temperature and longer treatment time (Kweon *et al*., 2000). Miyaguchi and Hu (2005) also reported that the solubility of *B. mori* silk fibroin increased sharply with higher concentration of $CaCl_2$, addition of ethanol and longer heating periods. The presence of sericin/gum and other impurities on the cocoon shells might also have prevented the penetration of the solvents and resulted in lower solubility of the cocoon shells than degummed fibers. The difference in solubility showed the variability existing in chemical structure and composition among the African wild silk cocoon shells and fibers.

The solubility of the African wild silk cocoon shells and fibers was significantly lower than *B. mori* except *G. postica* in LiBr solution demonstrating the presence of clear difference in chemical composition among the silks tested. Solubilisation of non-mulberry cocoons with LiBr and LiSCN proved also to be less effective and resulted in low yield than *B. mori* (Mandal and Kundu, 2008). Dissolution of a protein was reported to depend on its structure, molecular weight and structure of its macromolecules and polarity and steric arrangement of the side groups (Sashina *et al*., 2003). The amino acid sequence of polypeptides also plays a very important role in the solubility and crystallization of silk fibroins (Tanaka *et al*., 2002).

Fabrication of silk fibroin wastes produced by the silk industries involves formation of regenerated fibroin materials such as solution, powder, film, gel and filament by dissolving in proper solvents depending on its preparation conditions and application field (Sashina *et al*., 2006). Active efforts are made to develop processes involving preparation of working solutions of natural polymers and their conversion into particular, fibers and films. Although, the African wild silk is solely utilized as a textile raw material, the recent venture and development of silk in various areas of applications requires further processing such as dissolving in different solvent systems using several methods. Solutions of fibroin which can easily be transferred in to gels, powder or films for bio-material preparation are required. For this, solution systems which dissociate the intra-molecular bonding of silk fiber without breaking the polypeptide chains are essential. In this regard, even though this study is far from this confirmation, it sheds light on the solution properties of the African silk fibers and their potential for subsequent preparation of films, scaffolds and fibers for various applications.

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