Chemistry of Natural Compounds, Vol. 31, No. 4, 1995

THIN-LAYER CHROMATOGRAPHY OF THE SERICIN, FATTY WAXES, AND FIBROIN OF NATURAL SILK

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UDC 547.4.057

The method of thin-layer and high-performance thin-layer chromatography is proposed for the investigation of the isolation of sericin, fatty waxes, and fibroin from a natural silk cocoon. The chromatographic fractionation of fibroin according to molecular masses has been studied.

Natural silk consists of a fiber formed from microfibrils of fibroin cemented with sericin and containing fatty waxes [1]. In the process from cocoon to thread, the fiber is freed from sericin and fatty wax by washing in various solvents. The use of chromatographic methods (TLC, HPTLC) has permitted express information to be obtained on the molecular properties of both homogeneous and heterogeneous systems and to monitor the effectiveness of purification [2].

We have investigated the individual components of natural silk by the TLC and HPTLC method. It is known that the components of natural silk – proteins (sericin and fibroin) and also fatty waxes – contain various polar groups in their molecules [3]. Their chromatic separation requires the selection of mobile and stationary phases. The conditions of chromatography have been selected experimentally for each component in the light of the activity of the sorbent, the size of the particles, and the compatibility of the polarities of the eluent and of the substances to be separated. Acetic acid has been used as eluent [4].

Chemically pure microspherical silica gel obtained from tetraethoxysilane according to [5] has been used for the chromatographic analysis of molecules with different polarities. The optimum layer thickness of the sorbent on the plates is 0.05 cm.

The purity of the components of natural silk has been monitored by the TLC method, by the direct isolation of sericin and fatty wax from the solutions obtained in the washing with hot water, isopropyl alcohol, and petroleum ether of the cocoons freed from pupae (Table 1).

The value of R_f for the chromatographic spots of sericin is ~0.52; however, their area, S_s diminishes with time t, and after washing for 10 h it practically disappears. Analogous results were obtained in the determination of the sericin content, C, from the refractive index of the samples, n_D^{20}. This shows a constant decrease in the amount of sericin in the composition of natural silk as far as the complete freeing of the fibers from it.

Similar results have been observed in the determination of the amount of fatty wax S_f by the TLC method. On washing its soluble fraction in isopropyl alcohol, $R_f \approx 0.58$, the time of disappearance of the spot, t, is 10 h, while in the case of petroleum ether $R_f \simeq 0.56$ and $t = 6$ h. After the end of the washing of natural silk with solvents, the main substance remaining is fibroin. TLC analysis of the samples showed that R_f of fibroin dissolved in 2.5 M LiCl–DMFA is about 0.48 and its chromatographic spot is homogeneous and oval, which shows the achievement of a homogeneous protein system.

Fibroin molecules vary in their molecular masses and the volume dimensions of the chain. Their effective chromatographic separation is achieved with the optimum choice of stationary and mobile phases.

The sorbent with a particle size of 13-30 μm was replaced by one with a size of 3-5 μm, i.e., practically passing from the TLC regime into the HPTLC regime, and the chromatography of the fibroin was carried out. The eluent used was acetic acid, to which chloroform, and, in individual cases, benzene, was added in order to bring its pH to the isoionic point of fibroin. Under these conditions, when the polyampholyte property of the protein is screened, even with the optimum compositions of

Institute of the Chemistry and Physics of Polymers, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 44 26 61. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 588-590, July-August, 1995. Original article submitted October 17, 1994.

0009-3130/95/3104-0489$12.50

TABLE 1. Results of the TLC Analysis of the Isolation of Sericin and Fatty Wax from the Composition of Natural Silk

t, h	Sericin			Fatty wax	
	Water			Isopropyl alcohol	Petroleum ether
	S_c, cm^2	C, mt.%	n_D^{20}	S'_f, cm^2	S''_f, cm^2
1	0.85	33	1.3415	0.48	0.20
2	0.72	24	1.3395	0.45	0.13
3	0.59	15	1.3375	0.40	0.08
4	0.32	10	1.3360	0.35	0.04
5	0.15	8	1.3350	0.20	0.02
6	0.08	5	1.3345	0.09	0.01
7	0.05	2	1.3340	0.05	—
8	0.03	1	1.3335	0.02	—
9	0.01	0.2	1.3333	0.01	—
10	—	—	1.3330	—	—

TABLE 2. HPTLC Separation of Fibroin Molecules

R_f unfract.	R_f of the fractions	$\overline{M}\cdot10^{-3}$ of the fractions
0.11	0.16	106
0.33	0.40	158
0.69	0.65	275
0.82	0.81	378

the eluent, $CH_3COOH-CCl_4$ (7:3) and $CH_3COOH-C_6H_6$ (6:4), TLC analysis showed only an insignificant change of R_f in the direction of the start of the samples and an appreciable increase in the time of evolution of the spots. Complete separation of the spots took place in the HPTLC regime. Four spots differing in R_f values along the migration of the mobile phase on the plate were observed (Table 2). The results were compared with those of analogous experiments with samples of fibroin fractions obtained by fractional precipitation. It can be seen from Table 2 that the R_f values of the fractions from fractional precipitation were close to those from chromatographic fractionation.

The molecular masses of the fractions ($\bar{M}$) obtained by fractional precipitation were established by velocity sedimentation and by viscometry. An analysis of Table 2 permits the conclusion that the chromatographic separation of fibroin takes place with respect to molecular masses. Thus, to select the optimum conditions for purifying sericin, the fatty waxes, and the fibroin of natural silk, the method of TLC and HPTLC using as sorbents chemically pure microspherical silica gel with various particle dimensions and as eluents acetic acid and acetic acid with the addition of chloroform or benzene is proposed.

EXPERIMENTAL

Sericin was isolated from natural silk by washing the cocoons (15 g) with water (500 ml) at 90°C for 10 h. The water was replaced by pure water (90°C) every hour after samples had been taken for TLC.

The fatty wax was isolated by washing the purified sericin with isopropyl alcohol (500 ml) in a Soxhlet apparatus for 10 h. The part of the fatty wax extracted by ether was washed with petroleum ether (500 ml) in the same apparatus for 6 h. Samples were taken for TLC analysis every 1 h.

The fractional precipitation of the final washing product – purified fibroin – and the determination of the molecular masses of the fractions were carried out according to [6].

Microspherical chemically pure silica gel was synthesized from oligomeric tetraethoxysilane in the presence of isopropyl alcohol, glycerol, and ammonia [5]. The necessary particle size of the sorbent was obtained by fractionation with the aid of a RS-6 centrifuge, using a 5% aqueous suspension of the silica gel at a rotor speed of 1000 rpm. Particles with the required dimensions were selected according to time and were deposited on a 10 × 10 cm and in individual cases, 10 × 5 cm plates. The concentration of the silica gel suspension was 0.25 g/ml in a 2% solution of the oligomer in tetraethoxysilane.

TLC and HPTLC were performed under the same conditions with the addition of samples of the solutions of the specimens in volumes of 1 μl to a plate with a layer thickness of the sorbent of ~ 0.05 cm. The concentration of the sample solutions was ~ 0.03 g/ml.

The mass concentration of sericin (C, mass-%) was determined from the dry residue, and the refractive index n_D^{20} was measured on a IRF-22 refractometer.

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