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Thermochemical Study on the Dissolution and Regeneration of Fibroin from Solutions in Imidazole-Based Ionic Liquids

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Abstract—The enthalpies of dissolution of fibroin in imidazole-based ionic liquids were determined, and the contribution of polymer crystal structure decomposition to the enthalpy of dissolution was estimated. The heat of mixing of imidazole ionic liquid with water was described.

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Biopolymers (cellulose, chitosan, fibroin, keratin) are widely used in various fields of human activities. Processing of biopolymers gives rise to a lot of waste materials which may be reused, e.g., in the fiber spinning and film casting from concentrate solutions for domestic needs, technics, biotechnology, and medicine. A general property of the above biopolymers is their very poor solubility due to the presence of a large number of intermolecular bonds in the supramolecular structure. Appropriate solvents for the preparation of concentrated solutions may be aqueous and organic solutions of salts, *N*-methylmorpholine *N*-oxide, and ionic liquids on the basis of imidazole [1, 2]. The latter have been used as solvents for biopolymers relatively recently; therefore, some aspects of dissolution of natural polymers therein still remain unstudied; this is especially relevant to film-forming polypeptides such as fibroin. Nevertheless, the use of articles made of such polymers seems to be quite promising taking into account their compatibility with living matter, high strength, relatively high hydrophobicity, and resistance to bacteria. Fibroin and other polypeptides may be useful as materials for biochemistry, medicine, and biotechnology, for example, as fibers and films for wound repair and immobilization of enzymes and as many other articles.

There are very limited published data on the solubility of fibroin in 1-butyl-3-methylimidazolium chloride and acetate and on some properties of the solutions [2–4]. These data are insufficient to understand specificity of intermolecular interactions in the system fibroin–ionic liquid, effect of water on the dissolution and regeneration of the polymer from solution. In the present work we used thermochemical method to study solvent–solute interactions. Calorimetric parameters of dissolution make it possible to unambiguously describe processes occurring in a complex system, quantitatively estimate their energies, and compare the data for different solvents.

Figure 1 shows the plots of the enthalpies of fibroin (as washed silk fibers) dissolution in 1-butyl-3-methylimidazolium chloride (curve *1*) and acetate (curve *2*) versus concentration of the polymer in solution. It is seen that the enthalpy of fibroin dissolution does not depend on its concentration in the examined range. The dissolution of fibroin in 1-butyl-3-methylimidazolium acetate is accompanied by a higher exothermic effect as compared to 1-butyl-3-methylimidazolium chloride. Taking into account that the overall enthalpy of dissolution is the sum of some components: $\Delta H_{\mathrm{dis}} = \Delta H_{\mathrm{s-s}} + \Delta H_{\mathrm{p-p}} + \Delta H_{\mathrm{p-s}} + \Delta H_{\mathrm{dil}}$, where the first two terms characterize endothermic effect of bonds breaken between solvent and polymer molecules, respectively, $\Delta H_{\mathrm{p-s}}$ describes the exothermic effect of polymer–solvent interaction, and ΔH_{dil} is the enthalpy of dilution, the observed difference between ΔH_{dis} for 1-butyl-3-methylimidazolium chloride and acetate may rationalized as follows. First, the endothermic effect produced by bonds breaken between 1-butyl-3-methylimida-zolium acetate molecules to accommodate solvated polymer macromolecules is less considerable, for the solvent is liquid at room temperature (1-butyl-3-methylimidazolium chloride melts at ~60°C); second, the energy of interaction of 1-butyl-3-methyl-imidazolium acetate molecules with active centers of polymer molecules is higher. According to Hartree–Fock quantum-chemical calculations, the energies of interaction of 1-butyl-3-methylimidazolium chloride and acetate with fragments of cellulose macro-molecules are –24 and –28 to –31 kcal/mol, respectively [5].

The contribution of polymer crystal structure decomposition ($\Delta H_{\text{p–p}}$) to the enthalpy of dissolution may be fairly large, for natural fibroin is characterized by strongly ordered structure. It is known that *Bombyx mori* silk fibers consist of 56±5% of ordered β-sheet crystallites and 13±5% of molecules with α-helical conformation [6]; the remaining part of macromolecules or their fragments is disordered. Unlike α-helical structure, β-sheets are stabilized by intermolecular hydrogen bonds that are to be broken to release polymer molecule into solution. The helical conformation of macromolecules or their fragments is maintained by intramolecular hydrogen bonds which may be retained in solution. The contribution of decomposition of the ordered β-sheet structure to the heat of dissolution may be estimated by comparing the enthalpies of dissolution of the polymer as fibers and as films prepared by reprecipitation from solution. Our previous studies [7] and data of other authors [8, 9] allowed us to presume that fibroin macromolecules in dialyzed aqueous solution are mainly disordered and that they partly have α-helical conformation. The enthalpies of dissolution (ΔH_{dis}, J/g) of both samples of fibroin in the examined ionic liquids at 90°C are given below.

Fibroin	1-Butyl-3-methyl-imidazolium chloride	1-Butyl-3-methyl-imidazolium acetate
Natural fibers	–55	–195
Film	–75	–217

As might be expected, dissolution of less ordered fibroin film is accompanied by a higher exothermic effect, for the contribution of polymer structure decomposition is smaller. The contribution of breaking of highly ordered β-sheet crystallites may be estimated at ~21 J/g.

Water plays a specific role in the dissolution of polymers in hydrophilic solvents which include imidazole-based ionic liquids under study [10]. The presence of water in a solvent changes its properties; as a result, the solubility decreases, and the size of polymer particles in solution increases [11, 12].

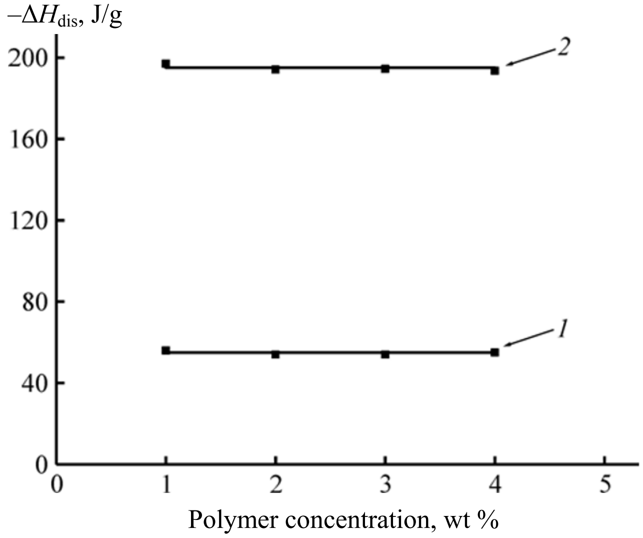

Fig. 1. Enthalpies of dissolution of silk fibroin at 90°C in (*1*) 1-butyl-3-methylimidazolium chloride and (*2*) 1-butyl-3-methylimidazolium acetate.

We have plotted phase diagrams for ternary mixtures fibroin–1-butyl-3-methylimidazolium chloride–water (Fig. 2, curve *1*) and fibroin–1-butyl-3-methylimidazolium acetate–water (Fig. 2, curve *2*). Solid lines delimit homogeneous solution from heterogeneous area. The unlimited mixing area for 1-butyl-3-methylimidazolium acetate is larger than that for its chloride analog due to higher dissolving power of the former. The critical water concentration (i.e., the concentration at which ionic liquid completely loses its dissolving power) is ~10% for 1-butyl-3-methylimidazolium chloride and more than 15% for 1-butyl-3-methylimidazolium acetate.

With a view to reveal specificity of the solvent–water interactions we analyzed the results of calorimetric measurement of the enthalpies of mixing of the ionic liquids with water (Fig. 3). Mixing of both ionic liquids with water was accompanied by a strong exothermic effect indicating formation of hydrogen bonds between the components, i.e., water competes with the polymer for solvation. This is likely to determine the influence of water on the dissolving power of ionic liquid. The maximal absolute value of the enthalpy of mixing for 1-butyl-3-methylimidazolium chloride is observed at a ionic liquid–water molar ratio of 1:1, and for 1-butyl-3-methylimidazolium acetate, at 1:2. Presumably, the reason is the chemical structure of anions in the ionic liquids. Chloride ion possesses only one donor center, whereas acetate ion provides two donor centers for hydrogen

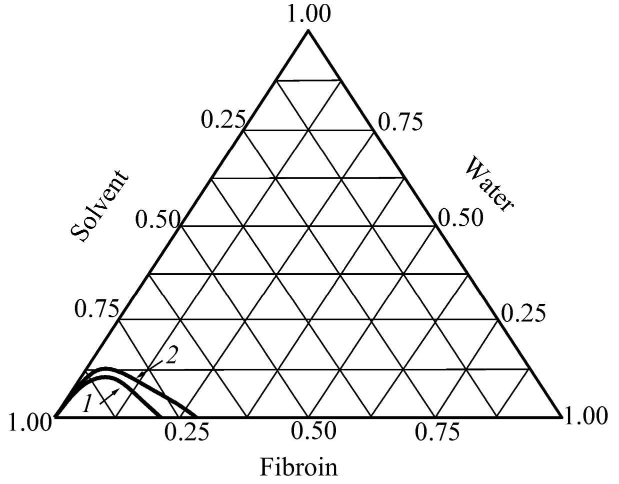

Fig. 2. Phase diagram of three-component systems fibroin–ionic liquid–water for (*1*) 1-butyl-3-methylimidazolium chloride and (*2*) 1-butyl-3-methylimidazolium acetate.

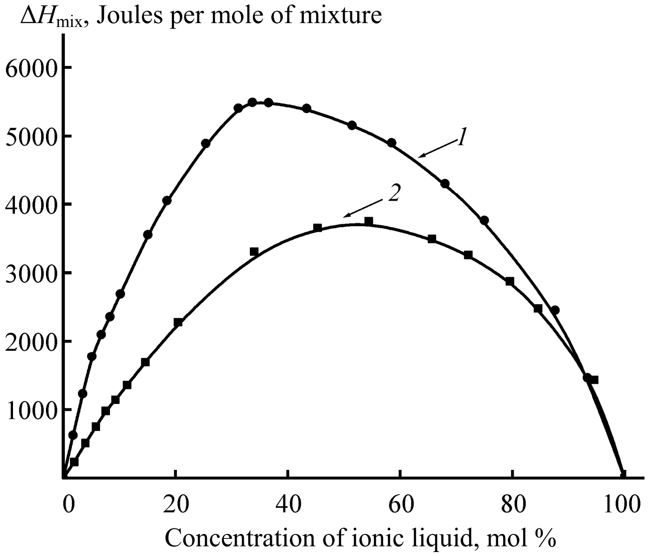

Fig. 3. Plots of the enthalpies of mixing of ionic liquids with water versus composition of the mixture at 90°C for (*1*) 1-butyl-3-methylimidazolium acetate and (*2*) 1-butyl-3-methylimidazolium chloride.

bonding with water. 1-Butyl-3-methylimidazolium acetate is capable of interacting with the polymer to a greater extent, as compared to 1-butyl-3-methylimidazolium chloride, upon addition of water which competes with the polymer for hydrogen bonding with the solvent.

EXPERIMENTAL

1-Butyl-3-methylimidazolium chloride and acetate were commercial products (from Merck). Fibroin from *Bombyx mori* silk fibers (as staple fibers 3.6 mm long and 34.6 μm in diameter) were washed off from sericin and fatty, waxy, and mineral substances according to the procedure described in [13] with 1% aqueous Na_2CO_3 and 1% aqueous sodium dodecyl sulfate (50°C, 1 h), followed by repeated washing with distilled water and drying. After the treatment, the average molecular weight of fibroin was 2.5×10^5. Fibroin films were prepared from a dialyzed solution of lithium bromide. For this purpose, fibroin was dissolved in a 6 M aqueous solution of LiBr at 40°C, the solution was cooled and poured into Spectra Por 1 Membrane MWCO tubings which were placed into distilled water and dialyzed for 24 h at room temperature with intermittent change of water. The dialyzed solution was poured onto Petri dishes and evaporated at room temperature.

The enthalpies of dissolution were determined at 90°C using a Setaram Calve calorimeter (France) equipped with a 10-cm^3 cell. The error in the measurement was ±5%. The concentration of fibroin in solution was no larger than 4 wt %; otherwise, the equilibration period considerably extended, and the experimental error increased, due to increased viscosity of solution.

Phase equilibria of fibroin-containing systems were studied by refractometry by measuring refractive indices of mixtures with different compositions provided that the difference in refractive indices of the components Δn was larger than 10^{-4} [14]. Insofar as homogeneous and heterogeneous areas are characterized by different dependences of the refractive index [15], the bent point on the dependence corresponds to a composition at which phase separation begins.

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