Preparation of Core–Shell Microcapsules Using Nanodispersed Fibroin

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ABSTRACT: An elaborate control of fibroin self-assembly is critical in determining the size, structure, and release properties of a microcapsule. This report describes the preparation of microspheres in the submicron range using an aqueous fibroin solution nanoemulsified in decane with a mixed detergent solution of Tween 80 and Span 80. Nanodispersed water droplets containing fibroin molecules were dried under vacuum to facilitate the coalescence of fibroin and then transformed to suspended particles in the range of 200–1200 nm in diameter. The method was also applied to encapsulate dextran and fluorescein isothiocyanate-dextran (FITC-dextran), producing core–shell structured microcapsules as identified by scanning electron microscopy and confocal scanning laser microscopy. The microcapsule yield was in the range of 82–85% per fibroin consumed. The encapsulation efficiency was 64% for the microcapsules fabricated from a nanoemulsion containing 0.1 mg of FITC-dextran per mg fibroin. Approximately 70.5% of the core material was released within 2 weeks. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 3460–3465, 2011

Key words: biopolymers; core–shell polymers; microencapsulation; proteins

INTRODUCTION

Silk fibroin from *Bombyx mori*, is an insoluble structural protein that has been used to prepare a variety of biomaterials for biomedical applications, including gels, sponges, and films.[1] Fibroin microspheres have been built by self-assembly and physical crosslinking of fibroin molecules to form a β-sheet structure, which is known to control the release rate of encapsulated enzymes.[2] Owing to their mild fabrication conditions, fibroin microspheres have been suggested as a platform for controlled drug delivery, being potentially advantageous for the encapsulation of sensitive drugs such as insulin-like growth factor-1 (IGF-I) released in a bioactive form over 7 weeks.[3] Various methods of microcapsule preparation by inducing fibroin gelation have been reported; these include sonication,[4] spray drying,[5] and freeze-thawing in ethanol.[6] Sonication-induced fibroin gel has been proven as useful for encapsulating mesenchymal stem cells, in terms of mechanical strength and cell proliferation with enhanced survival.[7] It has been suggested that the material properties of fibroin gel could be determined by the degree of β-sheet conformation self-assembled from a disordered state of fibroin molecules in solution.[8]

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In this report, we present a new method fabricating fibroin microspheres and microcapsules in the submicron size range. These were formed via coalescence and β-sheet formation among fibroin molecules solubilized as a nanoemulsion and harvested in the form of suspended particles in an oil phase. The formation of core–shell structured microcapsules encapsulating dextran is demonstrated and the release property is characterized.

EXPERIMENTAL

Materials

Silk fibers were degummed and solubilized to prepare an aqueous fibroin solution as reported previously.[9] Dextran in the molecular weight range of 35–45 kDa, fluorescein isothiocyanate-dextran (FITC-dextran) of molecular weight 40 kDa, and other reagents were purchased from Sigma-aldrich (St. Louis, MO).

Preparation of microspheres

A surfactant mixture (2 mL) of Span 80 and Tween 80 at a ratio of 5 : 3 (v/v) was dissolved in 16 mL of *n*-decane at 40°C and then 2 mL of 2% (w/v) silk fibroin solution was introduced into the mixed organic phase at room temperature. The mixture was emulsified using a homogenizer, (Polytron PT2100; Kinematica, Switzerland) at 26,000 rpm for 2 min.

Subsequently, the fibroin nanoemulsion was dried using vacuum rotary evaporator (JP/N 1000S-W; Eyela, Japan) at 40°C for 30 min. The samples were transferred to 50-mL conical tubes and centrifuged at 4000 rpm for 5 min. The precipitate was resuspended in ethanol, and the residual decane and detergents were washed with ethanol. For encapsulation, dextran or FITC-dextran was dissolved in 2% fibroin solution and the solution was introduced into the mixture of decane and detergents. The rest of the procedure was identical to that described above.

Microscopy

The prepared microspheres were resuspended in phosphate buffer (50 mM KH_2PO_4, pH 7.0) and dried on a slide glass or a cover slide. The samples were sputter-coated and observed by field emission scanning electron microscopy (FE-SEM) (*S*-4300; Hitachi, Japan). The resuspended microspheres were placed on a glass slide, and a cover slip was added and sealed. The slides were observed at 1200× magnification by confocal scanning laser microscopy (CSLM) (Fluoview FV300; Olympus, Japan). Optical images were obtained for each focal plane at 200-nm intervals with excitation of FITC at 488 nm.

Measurements

The droplet size of a nanoemulsion was estimated by dynamic light scattering using an electrophoretic light scattering spectrophotometer (ELS8000; Otsuka, Japan). Dried fibroin microspheres were analyzed using an FTIR spectrophotometer (Excaliber UMA 500; Bio-Rad, Hercules) and a thermogravimetric analyzer (SDT Q600; TA Instruments). To measure the release of FITC-dextran core material, microspheres were resuspended in phosphate buffer and incubated at 37°C for 20 days in the dark; samples were removed, and the fluorescence intensity was measured at 448-nm excitation and 506-nm emission using a fluorescence and luminescence reader (Fluoroskan Ascent FL; Thermo, Finland). The fluorescence intensity was converted to the corresponding concentration of FITC-dextran.

RESULTS AND DISCUSSION

Preparation of fibroin emulsions

It has been proposed that nanoemulsions can be prepared at an appropriate ratio of a detergent mixture of Span 80 and Tween 80 in a decane-water system.[10,11] In this report, similar emulsions were prepared as aqueous fibroin solutions in decane containing binary surfactant mixtures of Span 80 and Tween 80 at ratios of 8 : 0, 7 : 1, 6 : 2, 5 : 3, 4 : 4, 3 : 5, 2 : 6, 1 : 7, and 0 : 8 (v : v) at room temperature. The appearance of the emulsions of different fraction volumes of aqueous fibroin with the detergents at the various ratios was evaluated (Fig. 1).

Transparent emulsions were obtained at low volumetric fractions of aqueous fibroin solution. The aqueous fibroin solution was maximally solubilized up to 20% (v/v) in decane at a surfactant mixture ratio of 5 : 3 (v : v), corresponding to a value of 8.5 HLB. The transparent emulsions showed good stability over several weeks without any phase separation. Dynamic light scattering of a solution of 2.5% water fraction indicated the presence of droplets in the size range of 10 nm, corresponding to a volume of 5.24×10^{-25} m^3. As the average molecular weight of regenerated fibroin has been reported to be approximately 61.5 kDa,[5] the average amount of fibroin in each droplet corresponded to 1.7×10^{-25} mol, or 0.1 molecules per droplet. This suggests that the fibroin molecules were dispersed at the molecular level in the transparent emulsion.

Formation of fibroin microspheres

The elimination of the water droplets was expected to cause condensation of fibroin molecules in the nanoemulsion, because fibroin is not soluble in oil.

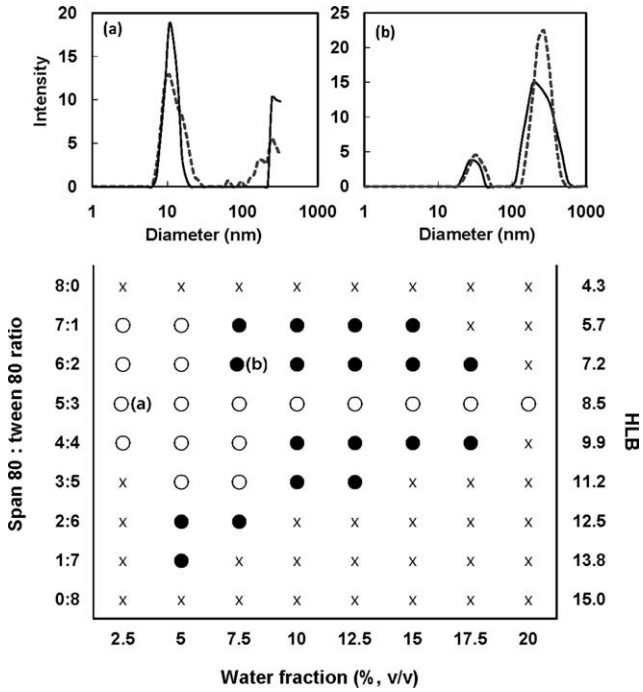

Figure 1 Appearance of emulsions of aqueous fibroin (2%) with Span 80 and Tween 80 at various ratios in decane. Lower panel: ○, transparent emulsion; ●, opaque emulsion; ×, phase separation. Upper panel: Microsphere size distribution in two preparations of emulsions (a) and (b), as determined by dynamic light scattering measurements.

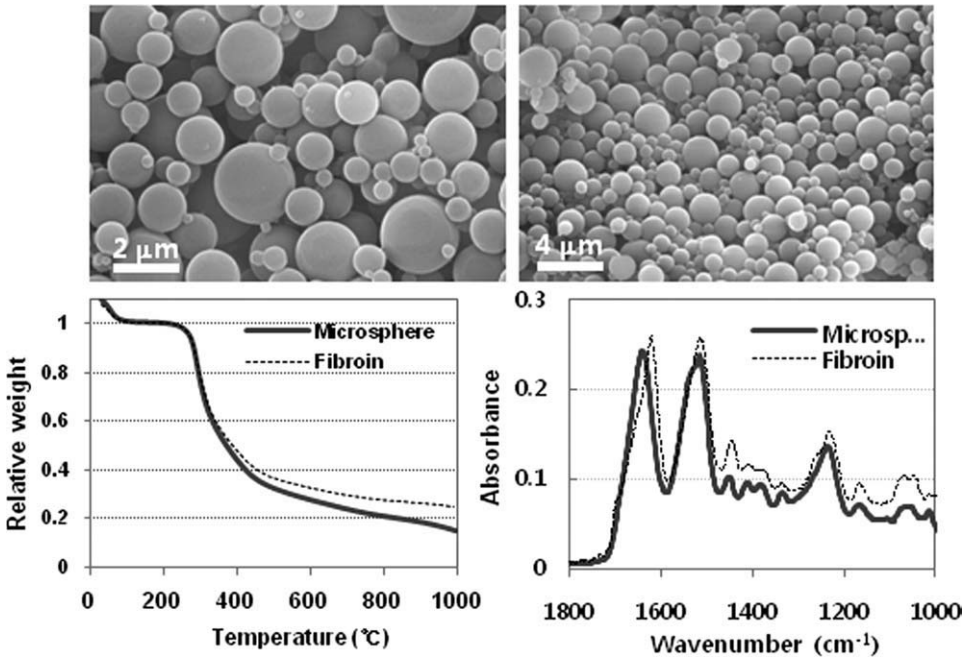

Figure 2 Scanning electron micrographs of fibroin microspheres at magnifications of ×10,000 and ×5000 (upper chart). Thermogravimetric and FTIR analyses of microspheres and fibroin (lower chart).

The transparent emulsion of 8.3 HLB value with a 2% water fraction was dried under vacuum, and the emulsion turned into an opaque solution. The suspended particles were recovered from the opaque suspension solution with 82.5% (w/w) yield per fibroin consumed. SEM analysis of the particles revealed smooth microspheres (Fig. 2). Thermogravimetric analysis showed that the moisture content of the microcapsules was 9% (w/w), and thermal decomposition profile was identical to that of fibroin except for a slight deviation above 500°C. The FTIR spectrum of the powder was similar to that of fibroin except for a shift of an absorption peak from 1643 to 1623 cm^{-1}. The shift of the amide I peak in the FTIR spectrum indicates a conformational transition of silk fibroin to a ß-sheet structure.[12] These results confirm that the particles were composed mainly of fibroin.

Fibroin molecules are unstable in aqueous solution and tend to aggregate as supramolecular globules or a gel.[13] Thus, fibroin microspheres have been fabricated by methods such as sonication[4] and shear stress[14] to facilitate condensation of fibroin molecules. This study demonstrates a new method for accelerated coalescence of fibroin molecules to form microspheres suspended in an oil phase. Vacuum dehydration of the nanoemulsion is expected to cause the formation of supersaturated fibroin molecules, which are responsible for particle growth. The surface tension of the decane phase accounts for the spherical form of the supramolecular assembly of fibroin.

Size distribution of fibroin microcapsules

The size of fibroin microspheres could be governed by the number of nuclei and the fibroin content in the transparent emulsion. Thus, the effect of homogenization speed for emulsion on the size of fibroin capsules was investigated. Figure 3 illustrates the size distribution of the microspheres prepared from emulsions homogenized at speeds of 19,000, 22,000, 26,000, and 30,000 rpm. The median microsphere diameters determined from the images were 730, 880, 610, and 600 nm, respectively. The higher homogenization speed (30,000 rpm) increased the fraction of smaller particles with diameters less than 600

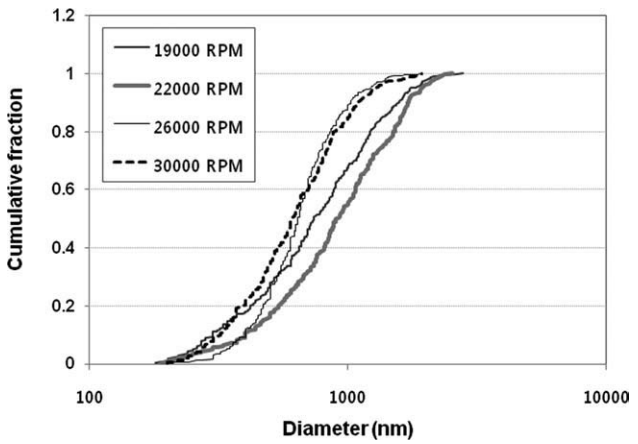

Figure 3 Cumulative size distribution of silk fibroin microspheres from emulsions prepared at various homogenization rates.

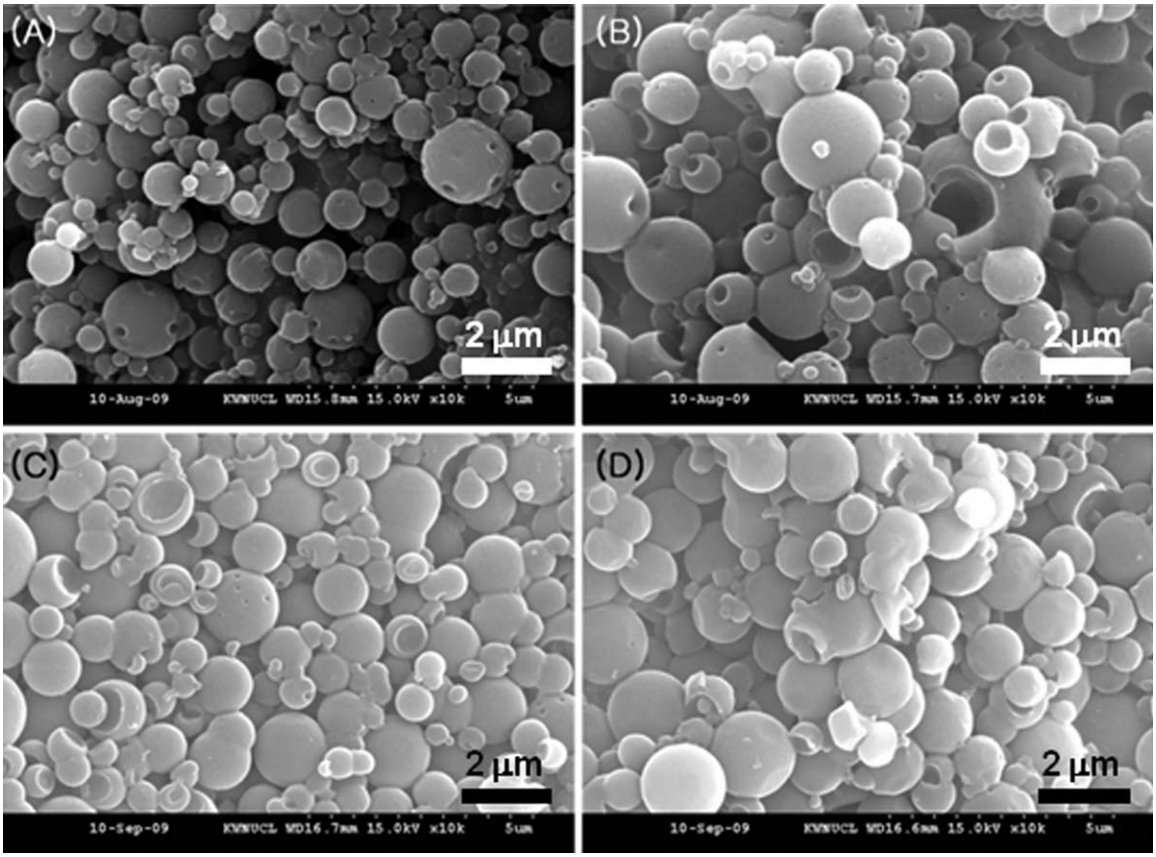

Figure 4 Scanning electron micrographs of fibroin microcapsules prepared using (A) 0.1, (B) 0.25, (C) 0.5, and (D) 1 mg of dextran per mg of fibroin.

nm, and approximately 90% of the microspheres had diameters between 200 nm and 1.2 μm. For further experiments, microspheres were prepared from transparent emulsions homogenized at 30,000 rpm.

Encapsulation of dextran and the internal structure

Dextran was chosen as a core material for encapsulation using the nanoemulsified fibroin. Various levels of dextran were solubilized in fibroin solutions, and microcapsules were prepared as described previously. SEM analysis indicated that the harvested microcapsules were mostly spherical, although small dents or holes were often found on the surface of the spheres, when dextran was supplemented at 0.1 mg per mg of fibroin (Fig. 4) and a greater number of dents or concavities were present on the surface of the microspheres formed with dextran greater than 0.25 mg per mg fibroin.

The internal structure of the microspheres was investigated by CSLM. Fluorescent rings were observed at each focal plane in the CSLM images of the microcapsules stained with FITC, indicating that the FITC-stained fibroin formed a shell structure surrounding an unstained dextran core [Fig. 5(A)]. To verify the core–shell formation, fibroin microcapsules were prepared with FITC-dextran as a core material, and CSLM revealed a shell structure of fibroin surrounding a fluorescent core [Fig. 5(B)]. Tomography showed single or multiple dextran cores in the center of the fibroin microcapsules.

The presence of multiple cores in the microcapsules suggests that the dextran did not serve as a nucleus for fibroin aggregation, and thus the dents on the fibroin microcapsules might have been caused by the discharge of dextran particles located close to the surface of the microspheres. The loss of dextran particles was expected to occur on resuspension of the microspheres in phosphate buffer, and a strong fluorescence signal was detected in the wash buffer. The presence of dents and cavities also indicates that dextran condensation occurred before the self-assembly of fibroin molecules and that subsequent physical cross-linking of fibroin molecules captured the dextran particles, forming a core–shell structure.

Release of dextran from fibroin microcapsules

The release property of fibroin microcapsules was investigated using microcapsules prepared from 80 mg of fibroin and 8 mg of FITC-dextran. Fibroin

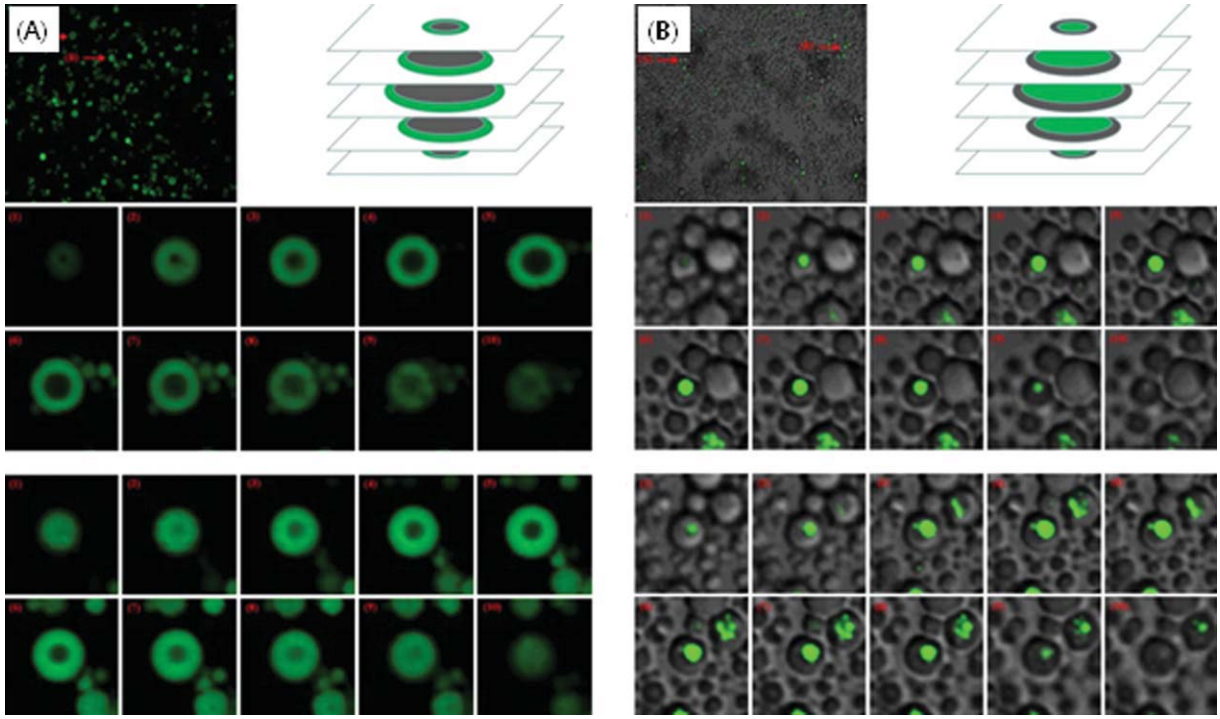

Figure 5 Confocal microscopy images and tomography of fibroin microcapsules prepared with FITC-stained fibroin (A) or FITC-dextran (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

microcapsules were harvested (75.1 mg) with 85.3% yield. Fluorescence measurements indicated that 2.88 mg of FITC-dextran had washed out during preparation; thus, 6.26 mg of FITC-dextran were retained in the 75.1 mg of fibroin microcapsules, with 64% encapsulation efficiency. The capsules were dispersed into 12 aliquots of phosphate buffer, and the release of FITC dextran was monitored. The fluorescence profile indicated that FITC-dextran was released over 2 weeks; the maximum level released, corresponding to 70.5% of the FITC-dextran encapsulated in the fibroin capsules, was attained at 16 days of incubation [Fig. 6].

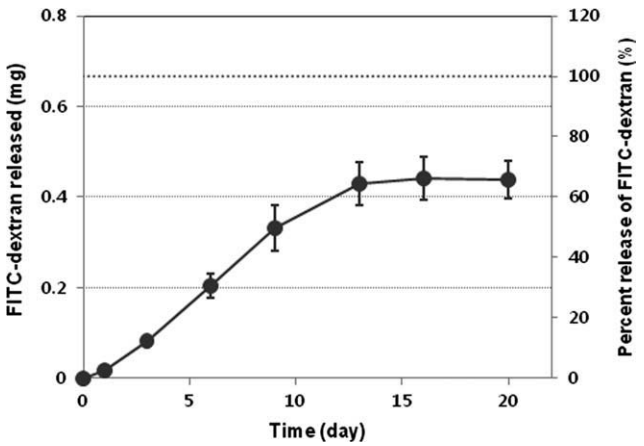

Figure 6 Release profile of FITC-dextran encapsulated in fibroin microcapsules in phosphate buffer at 37°C in the dark.

Fibroin microspheres have been prepared by various methods. Spray drying of fibroin solution has produced microspheres of 2 μm[5], and similar sized microspheres have been prepared using lipid vesicles as templates.[2] Larger spheres with diameters of 101–440 μm have been fabricated using laminar jet break-up of an aqueous fibroin solution.[7] By freezing fibroin solution with ethanol, microspheres ranging from 0.2 to 1.6 μm have been created.[6] Only the latter method fabricated fibroin microspheres with diameters in the submicron range, like those in this study; however, none of the previously reported fibroin microcapsules had a core–shell structure.

CONCLUSIONS

A new method for preparing fibroin microcapsules from fibroin dispersed at a molecular level is reported. The microspheres, with diameters between 200 and 1200 nm, were polydispersed. Intermolecular ß-sheet formation appears to be responsible for the particle growth, and spherical particles formed in the suspension owing to the interfacial tension of the oil phase. A core–shell microcapsule was fabricated by the encapsulation of dextran, and fluorescent dextran was released slowly over 2 weeks.

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