Results in Physics 5 (2015) 11–19

Contents lists available at ScienceDirect

Results in Physics

journal homepage: www.journals.elsevier.com/results-in-physics

An investigation on the chemical stability and a novel strategy for long-term stabilization of diphenylalanine nanostructures in aqueous solution

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ARTICLE INFO

Article history:
Received 24 May 2014
Accepted 6 December 2014
Available online 15 December 2014

Keywords:
Chemical stability
Diphenylalanine
Dissolution kinetics
Nanomaterials
Self-assembly

ABSTRACT

The stability of diphenylalanine (FF) microwires and microtubes in phosphate buffer solution was investigated and a novel strategy was developed for their chemical stabilization. This stability investigation was carried out by optical microscopy and by high performance liquid chromatography (HPLC). These microstructures dissolve in the solution depending upon their degree of FF saturation. The dissolution mechanisms of the structures in kinetically limited processes were found by accurately fitting the experimental dissolution data to a theoretical kinetic equation. The dissolution data were well fitted to the particular Avrami-Erofe'ev kinetic expression ($R^2 > 0.98$). These findings suggest that the structures can be stabilized by a decrease in the hydration of the constituent molecules thorough a chemical conformational induced transition upon heat treatment. The stable microtubes were fabricated in a novel three step procedure consisting of the reduction of silver ions in unstable FF microtubes by a citrate reductant, the stabilization by chemical conformational induced transition upon heat treatment, and the consequent oxidation of the reduced silver by a persulfate oxidant. These materials were characterized by electron microscopy and powder X-ray diffraction techniques. The long-term stability of both structures was also confirmed by optical microscopy and HPLC.

Introduction

Phenylalanine dipeptides (FF) are promising molecular building blocks for development of self-assembled nanostructures due to such attractive properties as biocompatibility, chemical versatility, functional flexibility, water solubility, and inexpensive synthesis [1–3]. These nanostructures were used extensively for rational design of various micro/nanomaterials. Among them, the micro/nanotubes (MNT) and micro/nanowires (MNW) were proposed for many current and emerging applications in nanobiotechnology owing to their unique properties arising from their anisotropic structure. The hollow tubular space of the MNTs provides the potential capability to be applied as nanotemplates/nanoreactors [4,5], catalyst supports/adsorbents [6], drug delivery systems [7], biosensing systems [8,9], and components in microelectronics [10]. The rigid MNWs can serve in composite reinforcement [11,12], energy devices [13], and biosensing systems [14–16]. These morphology dependant properties can be realized under a high degree of chemical stability in solution.

The chemical stability of the FF nanostructures was investigated in some research studies and methods were proposed for their stabilization. Andersen et al. [17] investigated the chemical stability of FF MNTs in seven different solvents ranging from organic ones to buffer liquids, including phosphate buffer saline. It is found that the MNTs immediately dissolve in unsaturated FF solutions. In their work, the proposed procedure for chemical stabilization is the formation of FF MNTs from supersaturated FF solutions at low acidic pH. This approach to stabilization will increase the synthesis duration from a few hours to a few days and decrease the density of FF MNTs on the unit surface. Ryu et al. [18] investigated the chemical, thermal, and proteolytic stability of the MNWs and the MNTs prepared by incubating FF amorphous film on solid substrates under aniline vapor at 423 K and under water vapor at 298 K, respectively. This solvent-vapor assisted self-assembly process resulted in the unstable MNTs and in highly stable hydrophilic diphenylalanine/polyaniline core/shell MNWs against chemical,

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http://dx.doi.org/10.1016/j.rinp.2014.12.001
2211-3797/

thermal, and proteolytic attacks. They studied the chemical stability of the MNWs by incubating them for 12 h in different solvents. The structural changes in the MNWs were then observed before and after incubation through scanning electron microscopy. Dissolution of the structures was not monitored continuously and the underlying mechanism of this process was not discussed as well. Demirel et al. [6] supposed that the formation of FF nanostructures is a question of solubility of the dipeptide monomers in different solvents, without any investigation on the chemical stability of the structures. The stability of nanostructures was increased by coating a thin film of poly (chloro-p-xylylene) onto FF MNTs. Adler-Abramovich et al. [19] and Shklovsky et al. [20] introduced the technique of chemical vapor deposition for the synthesis of chemically stable FF NTs. These vapor phase self-assembly processes are energy intensive and produce vertically aligned arrays of FF NTs on the solid substrates. The stability of the NTs was improved in these works by chemical conformational induced transition of the dipeptide from linear FF into cyclic FF upon heat treatment at 423 K. These studies all considered the stability of the FF structures without any discussion on the kinetics of dissolution. The dissolution mechanisms of unstable FF structures were not investigated in these studies as well.

In this study, the chemical stability of FF MNWs and MNTs in phosphate buffer solution was investigated by optical microscopy and by high performance liquid chromatography (HPLC). The dissolution kinetics of the structures in phosphate buffer was examined to understand the underlying mechanisms. The PB, a common biological buffer, is an appropriate medium to investigate the solubility of the dipeptide nanostructures in complex biological milieu. To the best of our knowledge, there is no published report on the dissolution kinetics of the FF structures in aqueous solutions. These studies led to the development of a novel strategy for fabrication of chemically stable FF MNTs and MNWs.

Materials and methods

Chemicals

Phenylalanine dipeptide monomers were purchased from Sigma (Sigma, St. Louis, MO, USA) in lyophilized form. HPLC solvents (acetonitrile and trifluoroacetic acid (TFA)/methanol) were bought from Sigma–Aldrich (Schnelldorf, Germany). All the other chemicals were purchased from Merck (Darmstadt, Germany) and used as received without any further purification. Chemical solutions were prepared with deionized water obtained from Avisa Shimi Teb Co. (Tehran, Iran).

Preparation of FF Micro/nanomaterials

Four kinds of FF micro/nanomaterials were prepared in this work, namely, the chemically unstable MNTs and MNWs and the chemically stable ones. The FF MNTs and MNWs were all formed from a stock solution of FF monomers. The stock solution was prepared by dissolving 100 mg of the FF powder in 25 ml deionized water at 353.15 K via sonication at 20 W. A fresh stock solution was prepared prior to each experiment to avoid premature aggregation. The stock solution was then diluted in deionized water to the concentrations of 3 and 2 mg/ml for the MNWs and the MNTs formation, respectively [21]. An aliquot of each solution was immediately poured into separate micro-wells that were fixed at a temperature of 288 K. This temperature shock from the solution temperature at 353.15 K to a temperature of 288.15 K induced rapid nucleation and produced fine and nearly uniform structures. The solutions were then aged for a day to complete the formation of the nanostructures. The suspensions were then dried using lyophilization at 223 K for 24 h in order to reach three-dimensional (3D) interwoven FF MNTs and FF MNWs. These native FF nanostructures are chemically unstable.

The chemically stable FF MNWs can be prepared by continuous heat treatment of both FF MNTs and FF MNWs in an oven (INE 400, Memmert, Germany) with a scan rate of 6 K min^{-1} up to a temperature of 423 K. The sample was then kept at this temperature for 30 min. This thermal treatment process turns the samples into the chemically stable MNWs as reported in previous research [22].

The chemically stable MNTs were prepared in a three step procedure. The freshly prepared stock solution was diluted in water to a concentration of 3 mg/ml. The diluted solution was immediately mixed with 200 μl of 0.1 M $AgNO_3$ and 200 μl of 0.1 M citrate solution. A 1 ml aliquot of the dipeptide solution was then deposited on a substrate and air dried at ambient condition until all the solvent was evaporated. The rapid evaporation process under the temperature shock increased nucleation process and led to a dense array of silver filled FF MNTs. In the second step, the MNTs were chemically stabilized under the same heat treatment process. Finally, the chemically stabilized MNTs were prepared by washing out the inner reduced silvers with 0.1 M ammonium persulfate solution for 30 min.

Online monitoring of the dissolution of FF nanostructures

To monitor the dissolution of FF MNTs and MNWs in a liquid environment, the micro/nanostructures were fixed to a substrate before introducing the phosphate buffer solution. To this end, a 100 μl aliquot of the dipeptide solution (2 mg/ml for MNTs and 3 mg/ml for MNWs) was poured onto the bottom of micro-wells and dried under atmospheric pressure at room temperature. The fixed nanostructures were placed under an Olympus CX21FS1 microscope (Tokyo, Japan) and continuously monitored with 10× objective lens as a 1 ml aliquot of 0.1 M phosphate buffer (PB) solution pH 7.0 was dispersed on top of the dried micro/nanomaterials. The reaction volume was fixed at 1 ml with time by time addition of the PB to compensate the vaporization effect. The effect of generated heat by the light on the solubility of the nanostructures was found negligible according to the reports of a previous study [17].

Dissolution rate measurement

The dissolution rate measurements of the native and chemically stabilized FF MNWs were carried out in the micro-wells in which the 3D micro/nanomaterials were synthesized. The physical properties of the samples were presented in Table 1. A 5 ml aliquot of the 0.1 M PB solution pH 7.0 was added to the micro-wells thermostated at ambient temperature (294 ± 1 K) in an incubator. The micro-wells were shaken at 150 rpm to ensure no mass transfer limitation to the bulk phase and to observe the real kinetics. The samples of 200 μl were withdrawn with micro-samplers at definite time intervals and filtered by a 0.2 μm filter. The filter, micro-sampler, and filter tanks were washed with deionized water several times and dried in an oven. The cleansed devices were kept at the experimental temperature prior to each test. The filtration

Table 1
Physical properties of the FF microstructures prepared in the form of three dimensional matrixes.

Sample	Density, g cm^{-3}	Porosity, %	Weight, mg	Average diameter, μm
FF MWs	1.272	99.7	3.35 ± 0.15	3.65
Stabilized FF MWs	NA[a]	99.8	2.8 ± 0.13	3.045
FF MTs	1.312	99.9	1.8 ± 0.1	1.708
Stabilized FF MTs	NA	NA	NA	1.103

[a] NA: Not available.