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

H. Nezammahalleh, G. Amoabediny, F. Kashanian, M.H. Foroughi Moghaddam

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 dependent 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, thermal, and proteolytic stress.
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 (chboro-p-xylene) 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 dissolusion 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 dipeptide 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 prepared by dissolving 100 mg of the FF monomers in 25 ml deionized water obtained from Avisa Shimi Teb Co. (Tehran, Iran).

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>
<thead>
<tr>
<th>Sample</th>
<th>Density, g cm$^{-3}$</th>
<th>Porosity, %</th>
<th>Weight, mg</th>
<th>Average diameter, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF MNWs</td>
<td>1.272</td>
<td>99.7</td>
<td>3.35 ± 0.15</td>
<td>3.65</td>
</tr>
<tr>
<td>Stabilized FF MNWs</td>
<td>NA$^4$</td>
<td>99.8</td>
<td>2.8 ± 0.13</td>
<td>3.045</td>
</tr>
<tr>
<td>FF MNTs</td>
<td>1.312</td>
<td>99.9</td>
<td>1.8 ± 0.1</td>
<td>1.708</td>
</tr>
<tr>
<td>Stabilized FF MNTs</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.103</td>
</tr>
</tbody>
</table>

$^4$ NA: Not available.
process was done at constant temperature to avoid the errors evoked by temperature fluctuations. The filtrate concentration was measured by the use of HPLC in a KNAUER system (Detection: UV at 257 nm; flow: 1 ml min\(^{-1}\)) and calculated by a calibration curve method. The extent of dissolution was determined by calculating the fractional dissolution (defined as the ratio of the concentration of dissolved sample to the initial concentration of the micro/nanostructures in the PB solution). The reversed phase chromatography was carried out using a C-18 column at an isocratic condition of 50% acetonitrile in 50% TFA (0.01%)/methanol. The dissolution rate measurements were done in triplicates to be sure of the reproducibility of the results.

**Instrumentation**

The SEM images of the FF MNWs and MNTs were recorded with a CamScan MV2300 (Electron Optic Services, Ontario, Canada) system after the samples were prepared on a microscope slide, 75 mm \(\times\) 25 mm, and coated with a thin layer of gold. The TEM images of the FF micro/nanomaterials were recorded with CM120 electron microscope (Philips, FEI Co., USA) at an operating voltage of 70 kV. To prepare TEM specimen, the water dispersed samples were dropped onto a carbon coated grid and dried in air at ambient temperature. The powder X-ray diffraction (XRD) measurements of the FF nanostructures were made using XPert MPD 2 \(\theta\) diffractometer (Philips Analytical, UK) with Cu anode, operating at 1.2 kW.

**Theoretical modeling of dissolution kinetics**

The reaction mechanism for dissolution of FF micro/nanomaterials consists of mass transfer of solvents into the reactant interface, formation of nuclei, the reaction interface advance, and mass transfer of reactant-solvent complex into bulk of the solution. Each of these processes can control the overall dissolution rate depending on the crystal shape and size distribution as well as on the type of solvent. Several theoretical models were developed in the literature to describe dissolution kinetics in crystalline materials [22,24]. These mechanistic models are characteristic of a crystalline system and depend on the crystal size distribution, shape, and constituents. These models were based on assumptions on which the rate of nucleation, reaction interface advance, and reactant-product diffusion to/from the interface were described. The dissolution reactions of the diphenylalanine nanostructures in agitated solutions are not under diffusion control and begin with instantaneous nucleation. The kinetic characteristics and the dissolution mechanism of this system can be understood by accurately fitting the experimental dissolution data to a theoretical kinetic expression. Those models applicable to the general kinetic characteristics of the FF crystalline system were reviewed in the following to find the accurate fit.

**Kinetic expressions for power law nucleation followed by constant rate of interface advance**

Kinetic behavior of dissolution reaction of solids is described by the calculation of fractional dissolution, \(\alpha\), versus time. The fractional dissolution can be predicted by finding the volume of all dissolved materials at time \(t\).

\[
V(t) = \int_0^t V(t, t_j) \left(\frac{dV}{dt}\right)_{t=t_j} dt_j
\]

(1)

where, \(\left(\frac{dV}{dt}\right)_{t=t_j}\) and \(V(t, t_j)\) are the rate of nucleation and the volume of the material occupied by a nucleus formed at time \(t_j\), respectively. The rate of nucleation can be described by an expression \( \frac{dV}{dt} = k_0 \beta^\beta \) [24]. Where \(\beta\) is the number of steps involved in nucleus formation (\(\beta = 0\) correspond to instantaneous nucleation, a value of one is the linear law of nucleation, and the values of \(n > 1\) are the power laws of nucleation). The occupied volume by each nucleus formed at time \(t_j\) can be described by the following law of nucleus growth [24].

\[
V(t, t_j) = \sigma(r(t, t_j))\beta
\]

(2)

where, \(\beta\) is the number of dimensions in which the nucleus grows and \(\sigma\) is a shape factor (for a spherical nucleus, it is \(4\pi/3\)). The length of each dimension, \(r(t, t_j)\), grows with a constant rate so that \(r(t, t_j) = k_p(t - t_j)\). The rate coefficient \(k_p\) can be different during dissolution time. The initial linear growth rate of nucleus is smaller than that measured later. Substitution of these expressions for nucleus formation and growth into Eq. (1) led to the following form.

\[
V(t) = \int_0^t \sigma k_p^\beta (t - t_j)^\beta \frac{dt_j}{k_p^\beta - 1} = \sigma k_p^\beta \left(1 - \frac{t}{\beta - 1}\right)
\]

(3)

where, \(V(t)\) is proportional to the fractional dissolution as

\[
\alpha = CK^\beta k_p^\beta t^\beta = Kt^\alpha
\]

(4)

where, \(\alpha\) is the extent of dissolution reaction ranging from 0 to 1. The \(C\) and \(K\) are the proportionality parameter and the dissolution rate constant, respectively. This dissolution kinetic is known as the power law and can present a correct description of dissolution kinetics in the early stages of the process. This equation is unrealistic due to continuous increase of the reaction rate up to an \(\alpha = 1\) for all values of \(n > 1\). In a real process, two common phenomena of coalescence and ingestion of nuclei exert a restriction on continuous nucleus development. Avrami [25] has provided a relationship between the so-called extended fractional decomposition (\(\alpha\), the extend of reaction which would have occurred if the two effects of coalescence and ingestion of nuclei had caused no limitation to the quantity of dissolved materials) and the actual amount which occurs in a real reaction, \(\alpha\).

\[
\alpha = \int_0^\infty \frac{dx}{1 - \alpha} = -\ln(1 - \alpha) = Kt^\alpha
\]

(5)

This expression is known as Avrami-Erofe‘ev equation and has been successfully applied for kinetic analysis of dissolution reactions.

**Kinetic expressions for rapid and dense nucleation followed by reaction interface advance from all or certain crystallographic surfaces into the bulk of the material**

The dissolution kinetics of such processes is determined by the geometry of advance of the reaction interface from these initially formed nuclei toward the center of the crystallites. In a contracting volume equation, the volume of reacted material from a cylinder with a diameter and length of \(r\) is obtained versus time. The rate of reaction interface advance from each face is supposed to be constant and equal, thus

\[
\alpha = \frac{\pi r^2 \pi - \pi(r - 2k_t t)^2 (r - 2k_t t)}{\pi r^2 r}
\]

(6)

where, \(k_t\) is the dissolution rate constant. After some algebraic simplification, the following contracting volume equation for kinetic analysis of dissolution process is resulted.

\[
1 - \frac{1}{r} = \frac{1}{k_t}
\]

(7)

If one dimension of a crystal is greater than the others, e.g. the length of a cylinder, the diminution in dimension of the crystal in this direction is small and approximately constant. This treatment of dissolution kinetics led to the following contracting area equation.

\[
1 - \left(1 - \frac{1}{r}\right)^{1/2} = \frac{1}{k_t}
\]

(8)

where, \(k\) in Eqs. (7) and (8) is the dissolution rate constant in \(\text{min}^{-1}\) and \(t\) is time of the reaction in minutes.
Fig. 1. SEM images of the FF micromaterials. (A) The native FF MTs; (B) the native FF MWs; (C) the heat treated FF MWs; (D) the composite FF MTs. (E-H) are the corresponding statistical distribution of diameters for the structures in (A-D). The inset shows high magnification SEM (A, B)/TEM (C, D) images of the corresponding structures.
Kinetic expression for chain type reactions

In chain type reactions, the initially formed nuclei yield intermediates of enhanced reactivity capable of activating new nucleation sites. The possible mechanism to these new nucleus formations could be either the development of cracks in the reactant phase or the development of needle or laminar nuclei which undergo branching. The rate of nucleation is expressed by the following equation [24].

\[
\frac{dN}{dt} = k_0 N_0 + k_B N - k_f(z)N
\]  \hspace{1cm} (9)

where, the first term in the right hand side represents the instantaneously formed initial nuclei, the second term is for constant rate of branching and the last one is the rate of termination due to coalescence or ingestion of developed nuclei. This rate of termination reaction is proportional to the fractional decomposition and the rate of branching. Prout and Tompkins [26] found a \( k_f(z) = 2k_B z \) relationship for the special case of symmetrically shaped sigmoid \( z \)-time curves with a point of inflection at \( z = 0.5 \). If the initially formed nuclei be much smaller than the ones formed later, \( k_0 N_0 \ll (k_B - k_f(z))N \), the following equation for nucleation law is resulted.

\[
\frac{dN}{dt} \approx k_0 (1 - 2z)N
\]  \hspace{1cm} (10)

They also assumed that the rate of dissolution is proportional to the number of nuclei so that, \( \frac{dc}{dt} = k N \). Combining Eq. (10) with this latter proportionality led to the following Prout–Tompkins equation for the dissolving native FF MWs (A) and the native FF MTs (B). Green arrows indicate completely dissolved crystals. The heat treated MWs (C) and the composite MTs prepared in the three step procedure (D) seemed stable. Scale bar corresponds to 200 \( \mu \text{m} \).
the thermal decomposition of potassium permanganate [26] which has also found application to the dissolution of crystals [24].

\[
\frac{dx}{dt} = k_0 x(1 - \alpha)^{\frac{1}{n}} \ln \left( \frac{x}{1 - \alpha} \right) = k t + c \quad \text{(11)}
\]

where, \(k\) is the dissolution rate constant in \(\min^{-1}\), \(t\) is time of the reaction in minutes, and \(c\) is a constant dummy parameter.

The actual fitting of the experimental data to the Eqs. 5, 7, 8 and 11 is done by plotting the left hand side of the equations as a function of time. The physical background of these equations was extensively reviewed and well presented in [24] as well.

Results and discussion

Chemical stability of FF nanostructures

The crystal geometry and size distribution in a suspension are influencing parameters on its dissolution kinetics. The MNWs and MNTs can be formed from FF solutions with different degree of supersaturation [21,27]. These materials have the same crystalline structure with variable morphologies depending on the degree of supersaturation [21]. These nanostructures are also similar to those prepared by dissolving the FF monomers in a fluorinated alcohol followed by dilution in water (see Fig. 1 in the supplementary information) [28,29]. Fig. 1A and B show both forms of the FF microstructures with the diameter distribution of each crystal in Fig. 1E and F, respectively. The stability of the materials was investigated in the 0.1 M PB solution pH 7.0 by observing the structural changes under the optical microscope immediately after the addition of solvent and by measuring the concentration of the dipeptide monomers in the solution. Fig. 2A and B shows the optical images taken at fixed time intervals during the dissolution process of MWs and MTs, respectively. The MWs and MTs that were fixed to the bottom of micro-wells experienced two phenomena upon the solvent addition. These native FF structures were found to slightly dissolve from sideways in the quiescent PB solution. It seems that smaller nanostructures, not visible in the view, dissolve rapidly and make a locally saturated environment for the larger microcrystals. This phenomenon would decrease the dissolution rate of the larger microcrystals.

To better monitor the dissolution kinetics of the structures, the concentration of the dipeptide monomers was measured by HPLC and optical microscopy, is mainly because of the locally FF saturated environment near the larger microcrystals (see Fig. 2 in the supplementary information).

The particular mechanism of dissolution of the FF structures in a kinetically limited process can be understood by fitting the experimental dissolution data to the theoretical kinetic equations. The dissolution rate of each structure was fitted to the \((\alpha_i, t_i)\) values in the early stages of the reaction (the acceleratory period) as the fitting to the data in the final stages is poor due to many experimental errors attributed to particle size, crystallite disintegration, and chemisorption of gasses on the residual phase. The correlation coefficient and the reaction rate constants for the Eqs. 5, 7, 8 and 11 are presented in Table 2. The given set of \((\alpha_i, t_i)\) for MWs and MTs obey the particular Avrami-Erofe’ev kinetic expression with time exponents \((n)\) of 0.66 and 0.67, respectively. This expression proposes that the dissolution begins immediately throughout the length of the microstructures and advances in one dimension \((\beta = 0 \text{ and } \lambda = 1)\), diffuses into the structures. The difference between these theoretical exponents of reaction (equal to 1) and the experimental value of about 0.7 can be contributed to the theoretical derivation of the equation that is not including all the processes involved in dissolution as well as to the experimental errors involved in the measurement. The lack of accurate fit to the Prout–Tompkins equation suggests no nucleus branching or crack development in the FN nanostructures during the dissolution. This understanding is in agreement with the experimental findings of Mason et al. [28] on the dissolution of FN nanostructures in aqueous solution. The dissolution rate of MTs is much higher than that of the MWs. This higher dissolution rate is caused by the hollow inside of the structure and better solvent accessibility. The heat treated MWs have shown a fixed and very low value of dissolution. This low dissolution rate is because of the FF monomers that are not joined to the structures and dispersed in the solution upon solvent addition. These dissolution results can be highly important in understanding the particular mechanism of dissolution of the FF structures in order to reach the solubility limit of the dipeptide monomers.

Table 2

<table>
<thead>
<tr>
<th>Theoretical expression</th>
<th>(R^2) for MWs</th>
<th>(k) (min(^{-1})) for MWs</th>
<th>(R^2) for MTs</th>
<th>(k) (min(^{-1})) for MTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avrami-Erofe’ev equ.</td>
<td>0.98</td>
<td>0.0852</td>
<td>0.99</td>
<td>0.5949</td>
</tr>
<tr>
<td>Contracting volume equ.</td>
<td>0.87</td>
<td>0.0065</td>
<td>0.74</td>
<td>0.0693</td>
</tr>
<tr>
<td>Contracting area equ.</td>
<td>0.84</td>
<td>0.0089</td>
<td>0.59</td>
<td>0.0882</td>
</tr>
<tr>
<td>Prout–Tompkins equ.</td>
<td>0.81</td>
<td>0.05669</td>
<td>0.87</td>
<td>0.3953</td>
</tr>
</tbody>
</table>

Fig. 3. Fractional dissolution versus time \((\alpha, t)\) data for isothermal dissolution of FF nanostructures in 0.1 M phosphate buffer solution pH 7.0 (measurement temperature is 294 ± 1 K). Dotted ellipses on the curve show the acceleratory period of dissolution process.

Fig. 4. XRD patterns of the native FF MWs (blue curve), the heat treated FF MWs (red curve), and the cyclic-FF powder (green curve).
the investigations on drug delivery systems based on FF nanostruc-
tures [7].

The results presented above suggest a strategy for chemical sta-
abilization by a decrease in the hydration of the FF constituents
(nucleus formation) and by a reduction in diffusion of the hydrated
FF chemicals into bulk of the solution. By reducing the mobility
of the FF constituents in the MTs formed at low pH, the MTs have
shown an increase in stability when submerged in an aqueous
environment [17].

Fabrication of chemically stable micro/nanowires

The FF nanostructures can be chemically stabilized in an aque-
ous solution by decreasing the solvent accessibility to the struc-
tures as well as by decreasing the solubility of the constituent
molecules in the solvent. The native MTs and MWs were composed
of multiple hydrophilic/hydrophobic nanochannels self-assembled
into one-dimensional supramolecular architectures with or without
a central lumen, respectively. These nanochannels can be
turned into needle like nanowires packed tightly together into
rigid MWs upon continuous heat treatment from ambient temper-
ature up to 423 K, as reported previously by Amdursky et al. [22].
The central lumen of the MTs is also closed during the thermal
treatment. This thermal process induces the chemical conforma-
tional change of the FF constituent molecules into cyclic-FF ones
with much lower solubility in aqueous solutions. The chemically
stable FF MWs were produced by continuous heating of the native
FF MWs up to a temperature of 423 K at a scan rate of 6 K min⁻¹.
This morphological transition accompanied by a change in the
 crystal structure of the native FF MWs was confirmed by powder
XRD analysis. The normalized diffraction patterns of the FF MWs
before and after heat treatment as well as that of the structures
made from cyclic-FF dipeptide are shown in Fig. 4. The XRD pattern
of thermally induced FF MWs is very similar to that of cyclic-FF
dipeptide and different from the one for native FF MWs. The chem-
ical stability of these FF MWs was investigated in the 0.1 M PB
solution (pH 7.0) by monitoring the structural changes under the
optical microscope immediately after the solvent addition as well
as by the continuous measurement of the cyclic-FF dipeptide con-
centration in the solution. Fig. 2C shows the optical images taken at
fixed time intervals during the dissolution process. The MWs seem
to be chemically stable in the PB solution due to lack of any struc-
tural changes over time. To confirm the results of the optical
microscopy examination, the concentration of linear FF and cyc-
lic-FF monomers were measured by HPLC at regular time intervals
during the dissolution process and the fractional dissolution were
calculated and plotted versus time in Fig. 3. This solubility investi-
gation by HPLC was done on 3D thermally induced FF micro/nano-
structures shown in Fig. 1C. The corresponding diameter
distribution of the crystals is shown in Fig. 1G.

![Fig. 5.](image-url) (Top panel) Schematic representation of the procedures involved in the hollow MT preparation (A). (Bottom panel) TEM image of the MTs before (B) and after (C) the
chemical oxidation of the reduced silver nanoparticles inside the MTs. Inset: TEM image of silver nanoparticle incorporated microtube.
Fabrication of chemically stable micro/nanotubes

The chemically stable MNTs were fabricated in a 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. The silver filled MTs were formed by the addition of 200 μl of 0.1 M AgNO₃ and 200 μl of 0.1 M citrate solution to the 3 mg ml⁻¹ dipeptide solution under continuous mixing. An aliquot of the solution was then left to be air dried on a substrate at ambient condition. These silver nanoparticle incorporated MTs were stabilized by the thermal treatment. This thermal process cannot close the central lumen of the MTs due to the reduced silver inside the hollow tubes. Consequently, the silver nanoparticles were oxidized and washed out from the inner space of the MTs. The chemical oxidation of the reduced silver was performed by 0.1 M ammonium persulfate oxidant under strong shaking for one hour. The exact procedure of the silver filled MTs is schematically presented in Fig. 5A. Fig. 5B and C shows the silver nanoparticle incorporated MT and the one left after washing out the silver nanoparticles, respectively. The silver nanoparticles are still present inside the porous structure of the tube due to the lack of the oxidant diffusion to the target sites (see Fig. 3 in the supplementary information).

This property suggests the possibility of increased conductivity and proteolytic stability of the structures. This property needs to be further investigated in the future researches.

The chemical stability of the MTs was investigated by optical microscopy and HPLC. A 100 μg of the dried MTs was dropped onto the bottom of microwells and physically fixed by lamellae. The stability was then investigated by dispersing a 1 ml aliquot of 0.1 M phosphate buffer (PB) solution pH 7.0 on the structures. The reaction volume was fixed at 1 ml with time by time addition of the PB solution to compensate the vaporization effect. Fig. 2D shows the optical images taken at fixed time intervals during the dissolution process. The lack of any structural changes of the MTs can be an indication of their chemical stability. The concentrations of the cyclic-FF and linear FF monomers were measured by HPLC at regular time intervals during the dissolution process and no noticeable peak relevant to the presence of the FF or cyclo-FF dipeptides was observed in the chromatogram. This solubility investigation by HPLC was done on 100 μg of the stabilized MTs that are shown in Fig. 1D with the corresponding diameter distribution of the crystals in Fig. 1H. The fractional dissolution was calculated zero in all measurements.

Conclusions

The stability of FF MTs and MWs in the PB solution was investigated in this study. These micromaterials are unstable and dissolve in the unsaturated solutions depending on the saturation level. In quiescent solution, slight structural changes were observed over time due to a locally saturated environment near the nanostructures while rapid dissolution was observed in a kinetically limited process. The dissolution mechanism of the structures obeys the particular Avrami-Erofe’ev kinetic expression. The dissolution rate constants of the structures were calculated as 0.085 and 0.595 min⁻¹, respectively, in the PB solution at 294 K shaken at 150 rpm. The dissolution rate of MTs is higher than that of the MWs because of increased solvent accessibility.

To realize the potential applications of the structures in agitated biological milieu, the FF MWs were chemically stabilized under a thermal treatment process which induces the chemical conformational change of linear FF constituent molecules into cyclic-FF ones with much lower solubility in aqueous solutions. The same idea was used for the fabrication of chemically stable FF MTs to prevent the morphological transition of the MTs into MWs during the heat treatment process, the hollow inside of the tubes was filled by silver nanoparticles prior to thermal treatment.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.rinp.2014.12.001.

References


