Rheological and micro-Raman time-series characterization of enzyme sol–gel solution toward morphological control of electrospun fibers

Dennis A Oriero, Andrew T Weakley and D Eric Aston

Department of Chemical and Materials Engineering, University of Idaho, PO Box 441021, Moscow, ID 83844–1021, USA
E-mail: aston@uidaho.edu

Received 26 January 2012
Accepted for publication 9 March 2012
Published 16 April 2012
Online at stacks.iop.org/STAM/13/025008

Abstract
Rheological and micro-Raman time-series characterizations were used to investigate the chemical evolutionary changes of silica sol–gel mixtures for electrospinning fibers to immobilize an enzyme (tyrosinase). Results of dynamic rheological measurements agreed with the expected structural transitions associated with reacting sol–gel systems. The electrospinning sols exhibited shear-thinning behavior typical of a power law model. Ultrafine (200–300 nm diameter) fibers were produced at early and late times within the reaction window of approximately one hour from initial mixing of sol solutions with and without enzyme; diameter distributions of these fibers showed much smaller deviations than expected. The enzyme markedly increased magnitudes of both elastic and viscous moduli but had no significant impact on final fiber diameters, suggesting that the shear-thinning behavior of both sol–gel mixtures is dominant in the fiber elongation process. The time course and scale for the electrospinning batch fabrication show strong correlations between the magnitudes in rheological property changes over time and the chemical functional group evolution obtained from micro-Raman time-series analysis of the reacting sol–gel systems.

Keywords: electrospinning, rheology, sol–gel chemistry, ultrafine fibers, nanofibers, micro-Raman, plateau modulus

1. Introduction
Electrospinning is a remarkably simple and highly versatile technique used in the production of a variety of one-dimensional (1D) nanostructured materials with diameters ranging from tens to a few hundred nanometers, within a short time [1–4]. It is a process that relies on electric charges (from an applied electric field) to deform a droplet of a fluid solution that is then ejected from a nozzle tip into ultrafine fibers [5]. Electrospun nanofibers have unique properties such as high surface area, potential for surface functionalization and modification, low weight, and high permeability making them suitable for diverse applications [2, 6]. These numerous applications include filtration, wound healing, tissue engineering scaffolds, drug delivery, sensors, energy storage/conversion devices, composite materials for reinforcements, sound absorption, heavy metal adsorption, protective clothing, catalyst and enzyme carriers, etc [2, 7].

The last decade has presented significant and promising research in the use of electrospun nano/ultrafine fibers as biocatalyst/enzyme carriers [8–12]. A novel research area in the fabrication of ultrafine fibers is the synthesis of hybrid/composite biocatalytic nano/ultrafine fibers using the combination of sol–gel chemistry with electrospinning. Such synthesis allows to produce a platform with a very high surface area for immobilizing active enzymes in a
Electrospinning with the enzyme already in the sol–gel solution presents several advantages compared to other conventional biocatalyst immobilization techniques, which are mostly physical and chemical methods. These advantages include bio-compatible/bio-friendly properties—due to the use of non-toxic reagents during the fabrication of the fibers and the biomolecular immobilization process occurring at ambient temperatures; the potentially easy addition of fiber porosity; the potential for reusability of enzyme-laden fibers that do not need to be recovered from free solution; low or no enzyme loss and leakage due to the immobilization on and/or within the fibers; and the inexpensive nature of electrospinning by comparison to many other techniques [10, 12].

However, there remains the significant challenge of rapid gelation, which hinders electrospinning silica fibers in batches on a lab-scale. The cross-linking reactions that transform the silica precursor sol into its much denser gel create the necessary three-dimensional molecular network to make the solid, sometimes porous fiber mat as the desired end product, which also renders electrospinning impossible at some point in the convention batch process. Oriero et al [12] produced a delaying effect on this rapid gel formation in the electrospinning process by adding a protic solvent (100 mM acetic acid) into the recipe during preparation of the enzyme-sol precursor. This provided a suitably long window of time to electrospin sufficient number of fibers for research purposes. The mechanism of this delaying effect is based on the formation of hydrogen bonds with reacting silanol groups to temporarily inhibit the cross-linking reactions (i.e., silanization) [13].

The designed delay in gelation opens a new practical time window for electrospinning with silica sol–gel chemistry; however, process still has the inherent potential for time-variant fiber morphology, specifically fiber diameter and—more important to future interests—porous formation targeted toward increasing active and accessible surface area for maximizing enzyme utilization. The fundamental factor is believed to be the changing viscosity of the electrospinning solution [1, 3, 5], or viscoelasticity, to be more broadly correct to the specific case of sol–gel electrospinning. The time-dependent viscoelastic behavior of the reacting fibers under formation has significant and at some times critical effect on fiber stability, final fiber diameter, and even pore formation. Detailed rheological characterization of the enzyme sol–gel system within the electrospinning time window will be required to understand the reasons for a particular fabrication outcome with respect to fiber mat morphology in terms of average diameter changes throughout a continuously-deposited fiber mat.

The present work attempts to assess the effect of evolving rheological and chemical properties of electrospinning silica sol–gel mixtures, with and without an active enzyme additive, on the final fiber diameters within randomly deposited layers during the course of the time window expanded by the judicious use of acetic acid [12]. Batches of fiber samples were characterized by scanning electron microscopy (SEM) to quantify fiber diameter distributions. Sol–gel mixtures were tested for elastic and viscous moduli by rheometry and for chemical functionality by micro-Raman spectroscopy over the same time window for electrospinning ultrafine fiber mats. The combined information is directed toward fine-tuning the electrospinning time against the desired fiber diameter and may also help establish optimum processing conditions for production scale-up, conversion from batch to continuous or semi-continuous electrospinning, better substrate coverage and uniformity, and ultimately for maximizing active and accessible fiber mat surface area for enzyme utilization in chemical detection and conversion processes.

For the production of porous fibers, merely targeting an increase in surface area does not necessarily mean it will be accessible for enzyme loading and activity or for enzyme–substrate processes to occur without substantial diffusion limitations on practical improvements. Smaller fiber diameters provide greater specific surface area externally, but exhibit a tradeoff in decreasing their superficial volume within which a porous network may be housed. The interplay between various electrospinning conditions and pore formation processes further complicates the situation, where the time window for consistent fiber diameter may not be over the same range or begin at the same time from initial solution mixing as the best time window for pore formation of a particular pore size distribution that would be beneficial for effective enzyme immobilization. A constant loading of the pore-forming agent D-fructose—as implemented in other studies [9, 12]—is included in these formulations as a fixed starting basis with the future plan of continuing parametric investigations toward optimizing pore formation for increased active enzyme loading. The present findings form the initial steps in a larger investigation to improve the properties of enzyme-fiber materials.

2. Materials and methods

Mushroom tyrosinase (polyphenol oxidase C. 1.14. 18.1) with a specific activity of 1460 units mg⁻¹ was obtained from Worthington Biochemical Corporation (Lakewood, NJ). Poly(vinyl alcohol) (PVA, 130000 mol. wt., degree of saponification 86.7–88.7%) and tetramethyl orthosilicate (TMOS, 99%) were purchased from Sigma Aldrich Chemical Company, Inc D-fructose was obtained from Fisher Scientific (Fairlawn, NJ). Sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrs (for preparation of sodium phosphate buffer, pH 6.8) and acetic acid were purchased from Mallinckrodt (Baker, NJ). The buffer solution pH was determined with a Dwyer PH-0-1 pH meter (Dwyer Instruments, Inc, Michigan City, IN). Glutaraldehyde (50% solution) was obtained from Polysciences, Inc (Warrington, PA) while hydrochloric acid was purchased from EMD chemicals (Gibbstown, NJ). Glass plates coated with indium-tin oxide on one surface (ITO-glass) were obtained from
from Delta Technologies (Stillwater, MN). An Advance Infusion Series 1200 syringe pump was obtained from Cellpoint Scientific Inc, Gaithersburg. Syringes (1 mL and 3 mL) and steel needles (27G 1/4 in.) were obtained from Becton, Dickinson (Franklin Lakes, NJ).

2.1. Preparation of enzyme sol–gel solution

The preparation of the enzyme (tyrosinase) sol–gel precursor solution for electrospinning was accomplished by an approach similar to that of Patel et al [10] with some modifications to make electrospinning of a sol–gel simpler and improve the stability of the fiber mat in aqueous environment. TMOS (0.76 g silica precursor), water (0.18 g for a H$_2$O : TMOS mole ratio of 2) and HCl (30 l of 40 mM stock as catalyst to speed up the hydrolysis reaction) were mixed under continuous stirring to form the hydrolized silica sol. Thereafter, the reaction mixture was heated to a temperature of 60°C for 30 min. The resulting sol was allowed to cool and the pore-forming material (500 l of 50% w/v aqueous D-fructose) was added with continuous stirring. PVA (700 l of 10% w/v) was then added to the mixture with continuous stirring. A cross-linker, gluteraldehyde, (200 l of 90 : 1 mole ratio gluteraldehyde : HCl) [14] was introduced to improve the mechanical stability of the fiber mat to be used in aqueous environments as reusable enzyme-immobilizing and potentially enzyme-encapsulating (within the pores) fibers. The addition of PVA, D-fructose and gluteraldehyde made the sol highly viscous, thereby facilitating electrospinning into fibers rather than electrospraying into droplets.

Fifteen l of 100 mM acetic acid were added to the electrospinning solution to delay gelation and provide a short but feasible time window for electrospinning. Tyrosinase enzyme (300 l) in buffer solution (6 mg mL$^{-1}$) was introduced into the above sol with continuous stirring for about 4–6 min before the mixture was electrospun into fibers. A second electrospinning sol was similarly prepared, adding 15 l of 100 mM of acetic acid, but without the enzyme, viz, silicate-fructose-PVA-glutaraldehyde.

2.2. Rheological characterization of the electrospinning enzyme sol mixture

Dynamic rheological measurements (oscillatory test/frequency sweep) were performed on the electrospinning sols with and without enzyme for analysis of elastic (storage) modulus (G'), viscous (loss) modulus (G''), loss tangent or tan(δ), complex modulus (G = G' + iG'') or complex viscosity (η = G' / G'') and steady-state shear flow over the sol–gel reaction time applicable for comparison to the electrospinning time window. Data obtained regarding these rheological parameters are important in understanding the sol–gel transition and the viscoelasticity of the sol and enzyme–sol in comparison; how these parameters relate to fiber diameters and distributions; and whether there is any correlation between these parameters and the effective electrospinning conditions for maximizing accessible surface area for nonwoven fiber networks and random mats. Rheological measurements were performed using a Bohlin CVO rheometer with parallel plate geometry (40 mm aluminum plates, 0.8 mm gap between the plates) at 25°C and with an applied oscillatory strain across a frequency range of 1–100 Hz. In addition, time-dependent studies were performed at a controlled frequency of 1 Hz for up to 1 h, equivalent to the longest feasible electrospinning duration of roughly 1 h.

2.3. Electrospinning fibers from enzyme sol mixture

Approximately 1 ml of the silicate-fructose-PVA-tyrosinase sol was introduced into a 3 ml syringe and placed in a syringe pump. The electrospinning setup [12] is similar to the one used by Jabal et al [15]; a syringe pump was used to control the sol flow rate at 10 l min$^{-1}$ and thus the droplet size undergoing electrodynamic distortion from a 10 kV source during production. The syringe containing the enzyme-sol mixture was subjected to an electric field of 100 kV m$^{-1}$ from the tip of the needle to the collecting electrode. The grounded collector plate (ITO-glass plate 25 × 18 × 1.1 mm$^2$, surface conductivity 0.010–0.014 $ S m^{-1}$) was placed at a distance of 10 cm from the needle tip. At the controlled flow rate, a continuous fluid jet resulting in the formation of ultrafine fibers randomly deposited onto the ITO surface as a white, nonwoven mat containing the enzyme tyrosinase [10]. Fiber mats without enzyme were produced in identical fashion. After the electrospinning process, the fiber mats were soaked in water for 2 h to attempt leaching of fructose from the fibers.

2.4. Morphological characterization of electrospun fibers

Average fiber diameters of electrospun fibers from electrospinning sol (with and without enzyme) within the allowable time window (up to 70 min) for electrospinning were imaged using SEM (Zeiss Supra 35 VP field-emission SEM, Center for Electron Microscopy and Microanalysis, University of Idaho). The fiber mats, electrospun onto ITO-glass plates, were sputtered with a thin layer of Au/Pd nanoparticles and then exposed to the electron beam. Thereafter, high-resolution (1280 × 1024 pixels) images were obtained for fiber diameter measurements using the ImageJ software (NIH). We measured 10 fibers per sample, 5 samples per group, and a total of four groups, or sample types, consisting of fibers with and without enzyme electrospun over early and late time windows, resulting in a sample-type distribution of 50 fibers represented by histogram. To ensure that a fiber within a given micrograph was not measured twice, a straight line was drawn along the image diagonal (using the line tool of the ImageJ software) and the fibers crossing this diagonal were labeled and measured.

2.5. Porosity and surface area measurements of electrospun fibers

The Brunauer–Emmet–Teller (BET) surface area and pore volume of the electrospun fibers were determined by N$_2$ adsorption-desorption isotherm measurements using a Micromeritics Tristar II 3020 physisorption analyzer. Before
measurement, the samples were degassed at 423 K and 500 mTorr for 3 h. Surface area, pore volume and diameter were calculated using the accompanying software from Micromeritics.

2.6. Micro-Raman time-series spectral analysis of electrospinning solution

Micro-Raman time-series spectra were acquired from samples of the reacting sols using a WITec alpha 300R scanning confocal Raman microscope (WITec GmbH, Ulm, Germany). Specifically, spectroscopic analysis was performed to monitor the real-time formation of siloxane via TMOS hydrolysis and condensation, the effects of acetic acid solvent-delay on gel formation and to make a connection as regards the timescale involved in electrospinning ultrafine fibers. Droplets of the electrospinning mixture were placed on a glass slide, and a Nikon 20× objective (NA = 0.4, WD = 3.8 mm) was used to focus the Raman excitation source (100 mW, 532 nm Nd : YAG laser) at the center of a droplet. Upon focusing, the sample was irradiated at a constant attenuated incident power of 8 mW for 75 min to prevent the thermal destruction of the sample during the study.

The Raman-scattered light was collected in the backscattering configuration and detected using a UHTS-300 spectrometer (WITec). A diffraction grating of 600 lines mm\(^{-1}\) was employed giving a spectral coverage of 200–3800 cm\(^{-1}\) of Raman shift. The integration time of the EM-CCD array detector was set to 5 s as the effective sampling rate of the reacting mixture, which resulted in 900 collected spectra for the 75 min time-series duration. Raman spectral data were processed and analyzed using the WITec Project software. Fluorescence baseline correction was performed using a third-order polynomial, followed by the application of a three-point moving average filter to eliminate most of the perturbing baseline. Following baseline correction, all spectra were scaled by the spectral average intensity. This procedure helped to mitigate the effect of large refractive index change on the intensities of the Raman bands over the course of the sol–gel reaction (clear-to-opaque liquid-to-solid material quality evolution). Of course, this procedure assumes that the refractive index change affects all bands equally.

Three Raman bands known to correspond to functional groups of interest (siloxane, silanol and methyl groups) were subjected to non-linear, least-squares curve-fitting procedure to estimate the amplitude changes of the Raman bands over the duration of the reaction, that is, in each of the 900 spectra per experiment. The band shapes were assumed to have a Gaussian profile, which is sufficient for the present study. When necessary, a five-point moving average filter was used to smooth all spectral bands prior to curve fitting. Plotting the estimated amplitudes of selected bands against time elucidates the time-scale of the major chemical features evolving in the sol–gel system.

3. Results and discussion

3.1. Time-dependent rheology and frequency sweep test

The typical log–log plots of elastic modulus (\(G'\)), viscous modulus (\(G''\)) and loss tangent (\(\tan(\delta)\)) with frequency (figures 1 and 2) and semi-log plots of complex modulus (\(G^* = G' + iG''\)), \(G'\), and \(G''\) values with sol–gel reaction time (figures 3 and 4) were generated from the two types of rheological experiments conducted. \(G^*\) may be thought of as an overall measure or index for general resistance to stress. Data obtained from shear flow analysis were fitted using the power-law fluid model (Ostwald–de Waele two-parameter model) \([15, 16]\): \(\sigma = K\dot{\gamma}^n\). The exponential factor \(n\) is the power-law index or flow behavior index, \(\dot{\gamma}\) is the shear rate, Power-law index values are less than one for
both electrospinning sols (0.47 without enzyme and 0.72 with enzyme; figure 5), reflecting a non-Newtonian, shear-thinning behavior: decreasing viscosity with increasing shear rate. The different quantitative behaviors with and without enzyme are affected in part by the general fluid properties of greater total fraction of dissolved and/or dispersed solids content (fructose + PVA + solparticles ± enzyme) and the fact that the enzyme is a higher molecular weight constituent being added, rather than a clear and specific physicochemical property of the enzyme alone. Shear thinning of the reacting sol mixtures occurs mostly due to the reduction in the rate of cross-linking of the three-dimensional poly-silicate network structure as the shear rate increases, resulting in a decrease in viscosity as well as slowing the emergence of the elastic behavior.

Log–log plots of $G'$ and $G''$ vs. frequency for sol mixtures with (figure 1) and without (figure 2) enzymes showed similar characteristics with $G'$ being dominant over $G''$ ($G' > G''$) from low to high frequency (1–100 Hz), typical of time-dependent viscoelastic materials like this polymer/reacting sol–gel solution. The characteristic features of viscoelastic fluids are observed in their oscillatory responses (figures 1 and 2); high phase angles (23° and 24° from $\tan(\delta)$ values 0.4245 and 0.4452, respectively) at low frequency (near 0 Hz, when the fluid is at rest), and generally lower phase angles at high frequencies. The loss tangent, as tangent of the ratio $G''/G'$, is a standard measure of the energy dissipation capacity of a material under cyclic stress compared to its elasticity [16–18]. Figures 1 and 2 also suggest that the sol–gel mixtures rapidly evolve into more solid-like behaving fluids, as expected with their gelation. Clearly, the sol mixture with enzyme is more capable of resisting stress by flow and elastic deformation than the sol without the enzyme.

The most significant feature is the presence of a rubbery or plateau region where the so-called plateau modulus ($G_p'$) can be determined as an index of the cross-link density in the network structure of the sol–gel mixture [19]. The cross-link density is a determinant of the rigidity of the gel. $G_p'$ can be calculated as

$$G_p' = \frac{cRT}{M_e},$$

where $c$ is concentration, $R$ is the gas constant, $T$ is the absolute temperature, and $M_e$ is the molar mass between cross-links [16, 19]. Values for $G_p'$ can be extrapolated from where $G'$ appears to have a flat or horizontal plateau (nearly zero slope), made more apparent on log–log scales. Within the plateau vicinity, $G''$ decreases with increasing frequency towards a minimum before rising again. Hence, $G_p'$ is determined as that value of $G'$ when $G''$ is a minimum and thus can also be easily deduced from $\tan(\delta)$ [16]. Larger values of $G_p'$ are then associated with greater gel strength [19]. Extrapolated $G_p'$ is almost an order of magnitude higher for the electrospinning sol with enzyme (1378 Pa) than without enzyme (163.2 Pa). It should be noted that equation (1) is not quantitatively rigorous unless the frequency state of this sol–gel is indeed behaving as an ideal rubbery solid. Although contradicted by the data, it is a fair approximation where the storage modulus is much greater than the loss modulus. The addition of enzyme clearly has a significant impact on the rheological properties of the sol and will affect the electrospinning process accordingly. Under the same applied electric field, it will be more difficult to electrospin the enzyme-sol, which can decrease the deposition rate and increase the fiber diameter but will also stabilize fluid jetting against breakup. This suggests that future experiments may be able to reduce the amount of PVA used in electrospinning with enzyme.

Figure 3 is the semi-log plot of $G'$, $G''$ and $G'''$ data for both electrospinning sol mixtures (with and without enzyme) as measured over approximately 6 min of reaction time, showing rapid, exponential increases in viscosity and elasticity. The elastic moduli over this early time window increase more quickly than the viscous moduli, and both properties are greater with the addition of enzyme. Data acquired at later times in the rheological scans exhibit regular, smooth trends only for up to roughly 15 min (Original data are reported in the supplementary data section, figures S1 and S2). After more than 15–20 min into the time-series measurements, rheology data become increasingly irregular or erratic with time for both sol types, and this is attributed to significant chemical structural changes in the evolving sol–gel, where the act of measuring rheology is in direct physical competition with the cross-linking process. From this observation, there is reason to suspect some characteristic shift in the electrospinning process and the final fiber quality between fibers produced at early and late times within a given batch. Fiber production rate is likely slower and the diameter and perhaps distribution would be increased.

It should be noted that the fairly rapid increase in viscoelastic resistance may also substantially affects the frequency-dependent studies illustrated by figures 1 and 2 while the increase in shear-thinning with frequency will somewhat compensate with respect to the magnitudes observed in figure 3 for constant shear at 1 Hz. Whether the net effect has a dominate bias over the results of figures 1 and 2.
Figure 4. Time-dependent rheological data for sol mixtures plotted from a weighted-residual method to smooth increasingly scattered data at late times.

Figure 5. Shear stress vs. shear rate graphs showing power-law fitted parameters for electrospinning sol mixtures (a) with and (b) without enzyme.

or is essentially self-canceling is very difficult to determine in a reacting system.

Figure 4 shows the time-dependent data for both source mixtures; the data are smoothed by a weighted-residual method (KaleidaGraph 4.1.1, Synergy Software) to display the average trend in rheological properties for convenience, without the inherent ‘noise’ at later times for the reacting sol–gel. Both figures 3 and 4 display similar, generally increasing trends in all parameters, with liquid-like behavior dominating initially ($G'' > G'$, figure 3) and solid-like behavior dominating at later times (figure 4) as expected for the gelling silica [16, 18–21]. Similar to the frequency sweep measurements, the magnitudes of $G'$, $G'$ and $G''$ are higher at all times for the enzyme–sol mixture than for the sol without enzyme. There is also a qualitative difference in the significantly higher rate of increase elasticity for the enzyme-gel material becoming obvious after 1500 s (figure 4). The elastic modulus may even decrease slightly over a few minutes of the testing for the gel without enzyme. This decrease may indicate that the enzyme has a significant structural impact on the growing gel network and a potentially stronger fiber product, though this hypothesis will need to be tested by some carefully designed mechanical measurement on the fibers.

3.2. SEM results

The average diameter (mean ± standard deviation, figure 6) of the fibers reflects the structural evolution of a sol–gel system as expected generally from the rheology results. Fibers from the early time window (zero up to ~25 min) have diameters about 60 nm smaller than those collected within the late time window (from 25 min up to ~1 h). The relatively large standard deviations suggest significant
Figure 6. Frequency distribution plots of the diameters of various samples groups of electrospun fibers indicate fairly broad ranges, consisting of 50 measurements per plot.

Even so, it is rather surprising in light of the rheology results that there is not a greater deviation in diameters from early to late times (figures 7 and 8). One would expect that the increasing viscosity and cross-link density would translate into increasing fiber diameters, but the shear-thinning quality, which would be substantial under the very rapid elongation regime of electrospinning the fluid into fibers, must be dominating the fluid-flow process in this regard, maintaining a roughly constant fiber diameter even within the early time regime where both viscosity and elasticity are increasing most rapidly. Furthermore, the enzyme-containing fibers are not statistically larger in diameter than their enzyme-lacking counterparts. Again, in spite of being unexpected on first consideration of the

Figure 8. SEM micrograph showing labeled fibers with enzyme electrospun within the late time window. The fiber diameters were measured using ImageJ software (Java based imaging program developed by the NIH).
3.3. Porosity and surface area measurements of electrospun fibers

The N\textsubscript{2} adsorption-desorption isotherm (figure 9) of the electrospun fibers is typical of a type IV isotherm with type H3 hysteresis loop (i.e. presence of a steep adsorption branch at relative pressures of unity and a sloping desorption branch at intermediate relative pressures) \[22\]. This hysteresis loop is associated with capillary condensation in pores with diameters of 2–50 nm, though the hysteresis is obviously very small. Type H3 hysteresis loops are also typically indicative of pores with open slit-shaped capillaries with parallel walls \[22\]. The average pore diameter (BJH pore diameter) of the electrospun fibers was 8.4 nm for the enzyme samples and 7.2 nm without enzyme (not shown). Though this pore size range is likely too small to avoid diffusion limitations for practical use as designed for enzymes, it suggests that the non-surfactant template method of pore formation in the silica sol–gel electrospinning process using D-fructose worked to some limited extent. The much lower than expected BET surface area of 2.9 m\textsuperscript{2} g\textsuperscript{−1} may indicate that the fructose has not been sufficiently leached out of the pores by soaking in water for two hours; for nanoporous material, we would expect a value somewhat smaller, but comparable to that of activated carbon, depending of course on the extent of porosity. Therefore, a new method may be needed to remove more fructose without damaging the fiber mats before use, which will be pursued in the future.

3.4. Micro-Raman time series spectral analysis of electrospinning solution

Figure 10 shows the baseline-corrected Raman spectrum acquired from the electrospinning enzyme-containing sol mixture after reacting it for 25 min, which is comparable to the electrospinning time (viz. post mixing). Figure 10 shows that the Raman spectral bands for three chemically reacting species associated with the sol–gel process were identified (i.e. normal modes of siloxane, silanol Si–O bond and methyl group) and their intensities were monitored during the time-series acquisition. Figures 11(a), (b) and (c) display snap-shots in the time-dependent peak intensities of these functional groups (CH\textsubscript{3} symmetric, Si–O antisymmetric and Si–O–Si symmetric stretching modes, respectively) at ten-minute intervals, as the reaction progresses \[23–26\]. Additional bands were labeled to illustrate a few points. For example, the intensities of the antisymmetric stretching modes of CH\textsubscript{3} and CH\textsubscript{2} moieties at wavenumber 2945 cm\textsuperscript{−1} (figure 10) remain steady in addition to the CH\textsubscript{3} antisymmetric deformation at wavenumber 1460 cm\textsuperscript{−1} \[26\]. PV A, fructose and gluteraldehyde all possess CH\textsubscript{3} and CH\textsubscript{2} groups whose Raman modes overlap within these regions. The symmetric carbonyl (C=O) stretch at 1655 cm\textsuperscript{−1} is explained by the formation of the ester functional group due to gluteraldehyde-PVA cross-linking reaction. The broad peak observed over 3000–3500 cm\textsuperscript{−1} reflects the inevitable presence of water in the mixture and originates from the OH groups (Si–OH inclusive).

Raman modes can be monitored for changes in peak intensity as a qualitative and relative measure of concentration for particular chemical functional groups according to their vibrational states. Figure 11(a) displays the time-dependent decrease in peak intensities of the CH\textsubscript{3} group (CH\textsubscript{3} symmetric stretching mode at wavenumber 2843 cm\textsuperscript{−1}), which originates from the precursor material TMOS and undergoes conversion to methanol (a significant percentage is lost via evaporation). This decrease is associated with the hydrolysis and condensation reactions. Figure 11(b) also presents a gradual time-dependent reduction in peak intensities associated with the Si–O bond from silanol. Si–O bonds are formed both in hydrolysis and condensation, which later progress into the formation of siloxane bonds \[13, 27\]. Figure 11(c) shows a steady time-dependent increase in the number of Si–O–Si bonds – the product of the cross-linking
and condensation reactions, which are responsible for gel formation.

Figure 11(d) is a convenient compilation of the time-dependent peak intensities of these functional groups as the reaction progresses. A decline in the band intensities of the Si–O moiety and CH$_3$ group is observed, while that of Si–O–Si increases during the process. The structural evolution of the sol into a gel is quite obvious, and the key time-series data as compiled in figure 11(d) allow direct comparison to rheological behavior with time. Due to the different drying conditions and material configurations between electrospinning fibers, reaction in the rheometer and under the Raman microscope system, it is difficult to conclude that the coincidence of the crossing of the Raman time-series intensities at ca. 30 min is quantitatively meaningful with respect to the obvious difference in fiber diameters spun before and after the 25 min mark. Negative intensities (not shown) are an inherent anomaly of background subtraction errors and have no physical meaning in an absolute sense; the negative-going drift with time of the methyl peak curve is indicative of the Raman mode disappearing into the background where the peak-fitting method becomes invalid. At $\sim$ 50 min, the amplitude of the Si–O band surpassed the detection threshold of the spectrometer, inhibiting the implementation of peak-fitting procedures. In the interest of continuity, the Si–O band profile was extrapolated empirically from 50 to 78 minutes using a third-order polynomial.

The acetic acid delaying effect on gelation appears in both rheometry and Raman spectrometry as a gradual but steady effect. While the downward trends in Raman peak intensities for the methyl and silanol moieties are obvious and expected (figure 11(d)), a more unusual feature is the gradual but clear collapse of the siloxane band (that is, the silica gel) beginning at $\sim$ 67 min, which might seem counter-intuitive on first consideration. This behavior was consistent across several experimental runs. This apparent weakening of the siloxane Raman mode probably reflects the tipping point in the physical/structural transition from a transparent suspension of the sol into the highly scattering translucent-to-opaque solid. From this time onward in the process, sufficient cross-linking of interconnected Si–O–Si bonds is already being formed in addition to the very low peak intensities of CH$_3$ and Si–O groups (indicating increased magnitude of condensation/poly-condensation and cross-linking reactions). These transitions are also in line with the fact that electrospinning fails at $\sim$ 1 h (similar time range of rheology results) due to hard gelation overcoming the electric field.

4. Conclusions

The optimum time window for electrospinning the tyrosinase-encapsulating porous silica gel fibers with relatively high surface area was identified to be within $\sim$ 25 min after preparing the sol mixture, which yields smaller fiber diameters due to lower cross-link densities at the time of spinning. The acetic acid delaying effect on silica
gelation appears in both rheometry and Raman spectrometry as a gradual but steady affect. Fibers produced after 25 min from mixing are thicker by 60 nm on average than the early time fibers. With the current formulations, the practical time window for producing sol–gel fibers is restricted to an hour due to hard gelation halting the electrojetting process. To determine a more specific optimum time window, the balance must be set between production rate, or amount (e.g. fiber mat thickness and collector substrate dimensions), and required specific surface area, porosity or fiber diameter.

The shear-thinning quality of sol mixtures with and without enzyme appears to dominate in the electrospinning process rather than the elastic and/or viscous moduli values regarding fiber diameter, since there is no statistically meaningful difference in fiber diameters of both types. The larger plateau modulus and higher viscosity of the enzyme-containing sol–gel system support the possibility of reducing the amount of PVA required to maintain stable electrospinning, which should reduce the final fiber diameter to increase accessible surface area in the mats. There is some hint from elasticity data in the late time window that the addition of the enzyme may in some way increase the final fiber strength without impacting fiber diameter.

Acknowledgments

The authors gratefully acknowledge the support of the National Science Foundation (award DMR-0619310), the United States Department of Agriculture (award no. 2009–34479-19833), the USDA National Institute of Food and Agriculture (grant no. 2010–34479-20715) and the University of Idaho Biological Applications of Nanotechnology (BANTech) Center; technical assistance from Thomas J Williams and the electron microscopy center (University of Idaho); help with construction and assembly of the electrospinning voltage source and collector metal frame from Dr David MacPherson and Mr Charles Cornwall (Chemical & Materials Engineering, University of Idaho), respectively; expert training and technical advice in rheometry from Mr Lance Gallagher and Professor Armando McDonald; and expertise and access to BET measurements at Washington State University from Stephen Davidson and Professor Yong Wang in The Gene and Linda Voiland School of Chemical Engineering and Bioengineering.

References

[1] Teo W E and Ramakrishna S 2006 Nanotechnology 17 R89
[2] Fang J, Niu H, Lin T and Wang X 2008 Chin. Sci. Bull. 53 2265
[3] Ramakrishna S, Fujihara K, Teo W, Lim T and Ma Z 2005 An Introduction to Electrospinning and Nanofibers (Singapore: World Scientific)
[4] Burger C, Hsiao B S and Chu B 2006 Annu. Rev. Mater. Res. 36 333
[5] Li D and Xia Y 2004 Adv. Mater. 16 1151
[6] Sawicka K M and Gouma P 2006 J. Nanopart. Res. 8 769
[7] Thavasi V, Singh G and Ramakrishna S 2008 Energy Environ. Sci. 1 221
[8] Sawicka K, Gouma P and Simon S 2005 Sensors. Actuators B 108 585
[9] Ren G, Xu X, Liu Q, Cheng J, Yuan X, Wu L and Wan Y 2006 React. Funct. Polym. 66 1559
[10] Patel A C, Li S, Yuan J and Wei Y 2006 Nano Lett. 6 1042
[11] Arecchi A, Scampicchio M, Drusch S and Mannino S 2010 Anal. Chim. Acta. 659 133
[12] Orio et al. 2009 Sensors. Actuators A, Rouquerol J and Siemieniewska T 1985 Anal. Chim. Acta.
[13] Hench L L and West J K 1990 Chem. Rev. 90 33
[14] Tang C, Saquing D C, Harding J and Khan S A 2010 Macromolecules 43 630
[15] Jabal J M F, McGarry L, Sobczyk A and Aston D E 2009 ACS Appl. Mater. Interfaces 1 2325
[16] Barnes H A 2000 Handbook of Elementary Rheology (Aberystwyth: University of Wales Institute of Non-Newtonian Fluid Mechanics)
[17] Rodriguez F, Cohen C, Ober C and Archer L 2003 Principles of Polymer Systems (New York: CRC Press)
[18] Tanner R I 1985 Engineering Rheology (Oxford: Clarendon)
[19] Böhm N and Werner-Michael K 1999 Carbohydr. Res. 315 302
[20] Sacks M D and Sheu R 1987 J. Non-Cryst. Solids 92 383
[21] Friberg S E, Jones S M, Motyka A and Broze G 1994 J. Mater. Sci. 29 1753
[22] Sing K S W, Everett D H, Haul R A W, Moscic L, Pierrotti R, A, Rouquerol J and Siemieniewska T 1985 Pure Appl. Chem. 57 603
[23] Schrader B 1995 Infrared and Raman Spectroscopy: Methods and Applications (New York: VCH)
[24] Baranska H, Kabudzinska A and Terpinski J 1987 Laser Raman Spectroscopy: Analytical Applications (New York: Wiley) p 138
[25] Colthup N B, Daly L H and Wiberley S E 1990 Introduction to Infrared and Raman Spectroscopy (New York: Academic)
[26] Miller F A, Mayo D W and Hannan R W 2003 Course Notes on the Interpretation of Infrared and Raman Spectra (New Jersey: Wiley) p 15
[27] Brinker J C and Scherer G W 1990 The Physics and Chemistry of Sol–gel Processing (San Diego, CA: Academic)