Helical poly-L-glutamic acid templated nanoporous aluminium oxides†

Jeng-Shiung Jan and Daniel F. Shantz*

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The synthesis of porous aluminium oxide made in the presence of helical poly-L-glutamic acid is reported.

Developing new synthetic approaches to fabricate porous oxides with unique and controllable structural features is currently of great interest. In this regard the use of biological molecules such as amino acids, peptides, and proteins has drawn considerable attention.[1–6] Materials such as silica diatoms and nacre are held out as the ultimate examples of the complex structures nature can assemble at ambient conditions. In many regards these goals have energized the field of inorganic materials chemistry and have been summarized in several excellent reviews.[7–9]

Recent work in our laboratory has taken a different approach, namely using the folded state of a polypeptide chain (*e.g.* α-helix) or polypeptide aggregate (*e.g.* β-sheet) as a means to tailor pore architectures. In our initial report[10] we describe the synthesis of silicas wherein the pore size and shape are a consequence of the folded state of the poly-L-lysine used as the template. Here we demonstrate, using aluminium oxide, that this is also possible for the synthesis of oxides at low pH (<3) using helical poly-L-glutamic acid. These materials, while amorphous by X-ray diffraction and transmission electron microscopy (TEM), are porous after the removal of the polypeptide by extraction or calcination. *In situ* circular dichroism measurements demonstrate that the polypeptide observable by CD does not unfold during synthesis, and IR spectroscopy indicates that the polypeptide in the as-made composite retains its folded state. This work demonstrates the general applicability of the concept that the folded state of a polypeptide chain, here in a helical state, can be used to achieve a templating effect.

Poly-L-glutamic acid (PLGA, Na^+-salt, MW ~ 17,000) and $AlCl_3 \cdot 6H_2O$ were used as received from Aldrich. Oxides were synthesized by using 0.1 N HCl solution to adjust a 0.5 mg ml^{-1} solution of PLGA to pH 4. After 15 minutes of mixing this solution a 0.1 M metal chloride solution was added. Precipitates were formed within 30 minutes, which were collected by centrifugation, filtered with copious quantities of deionized water, and then air dried overnight. The polypeptide was removed from the oxide by calcination at 673 K for 8 hours. FE-SEM images of the samples after removal of the peptide indicate that small particles, of approximately 50 nm in size, are formed. Both powder X-ray diffraction and TEM verify that the materials are amorphous and possess no long-range structural order.

Fig. 1 shows an FE-SEM image and α_s plot[11] for the PLGA templated alumina after calcination. The surface area of the aluminium oxide is 340 $m^2\ g^{-1}$. Using non-porous aluminium oxide C as the reference material,[12] the α_s analysis for the alumina sample shows the total pore volume to be 0.19 $cm^3\ g^{-1}$. The tangent line of the α_s curve taken from $1.0 < \alpha_s < 1.2$, yields a micropore volume of 0.1 $cm^3\ g^{-1}$. The isotherm does not exhibit a hysteresis loop excluding the presence of pores > 4.0 nm. While a lack of suitable reference materials makes calculating the pore size distribution ambiguous at best from the adsorption data, it is clear from the α_s analysis that, if the PLGA remains in a helical conformation (see below), the pores formed are due to individual PLGA chains or PLGA dimers. The supporting information also contains the α_s plots for the analysis performed using a non-porous α-alumina as the reference material.† BJH analysis of the adsorption isotherm shows two maxima at approximately

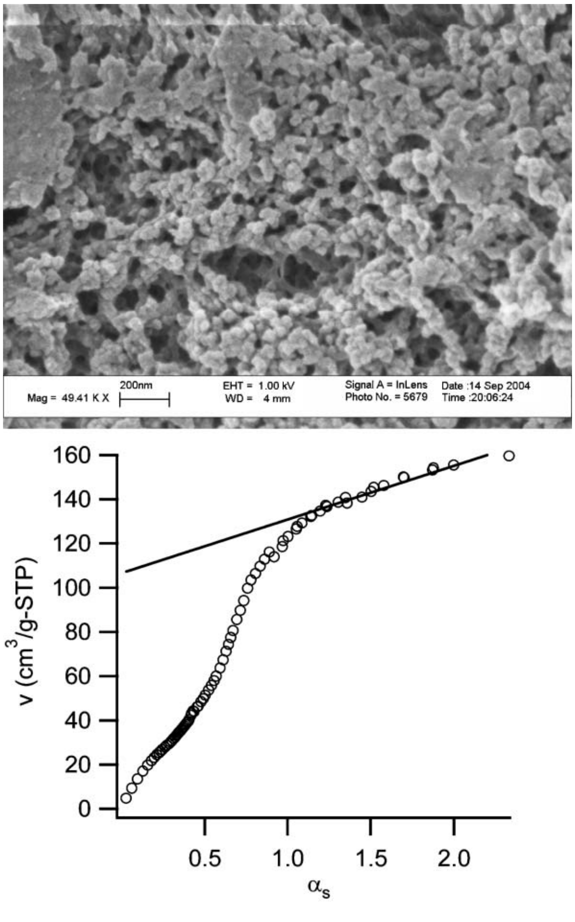

Fig. 1 (Top) FE-SEM image and (bottom) α_s plot for PLGA templated alumina. Scale bar in the FE-SEM image is 200 nm.

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† Electronic supplementary information (ESI) available: FE-SEM, TEM, *in situ* solution CD, IR, nitrogen adsorption, and α_s-plot of PLGA-templated iron oxide phase. α_s-plot of PLGA-templated alumina using α-alumina as the reference material. See http://www.rsc.org/suppdata/cc/b4/b416113k/

*shantz@che.tamu.edu

1.5–2.0 nm and 3.0–3.5 nm depending on the form of the expression for the statistical film thickness, *t*. The validity of the BJH formalism is questionable for pores in this size range,[11] however the results are qualitatively in agreement with the α_s-analysis.

The nitrogen adsorption data clearly shows the materials are porous, however it cannot provide quantitative information regarding the pore size distribution. Hence we performed two additional sets of experiments to probe the conformation of the poly-L-glutamic acid *during* oxide synthesis and the polypeptide conformation inside the as-made hybrid material. Fig. 2 shows *in situ* circular dichroism (CD) measurements. Upon addition of the metal chloride to the PLGA solution there is a decrease in the signal intensity, however the transition from an α-helix → random coil is clearly not observed. In all spectra the double minima indicative of an α-helix are clearly observable; by contrast the positive ellipticity at 220 nm indicative of a random coil is not observed. The decrease in signal intensity is consistent with the observation that large oxide particles form very rapidly. Hence one would expect a decrease in the overall signal intensity given that the path length (*i.e.* cuvette size) is the same for all spectra shown. These measurements show that the polypeptide chains observable by CD are in a helical conformation.

Fig. 3 shows the IR spectra of the as-made hybrid material. While the bands are very broad compared to solution spectra of PLGA, the IR of the as-made PLGA–alumina composite material is consistent with helical PLGA. The bands at 1654 cm^{-1} and 1543 cm^{-1} are consistent with previous work[13] and indicate a helical conformation, and the shoulder at 1720 cm^{-1} is also consistent with the side-chain carboxylate group being protonated. The spectrum is consistent with the polypeptide retaining a helical conformation inside the oxide. The CD results show clearly that the PLGA observable in solution is helical. The IR results for the alumina samples support that the helical conformation of the PLGA is retained in the hybrid material.

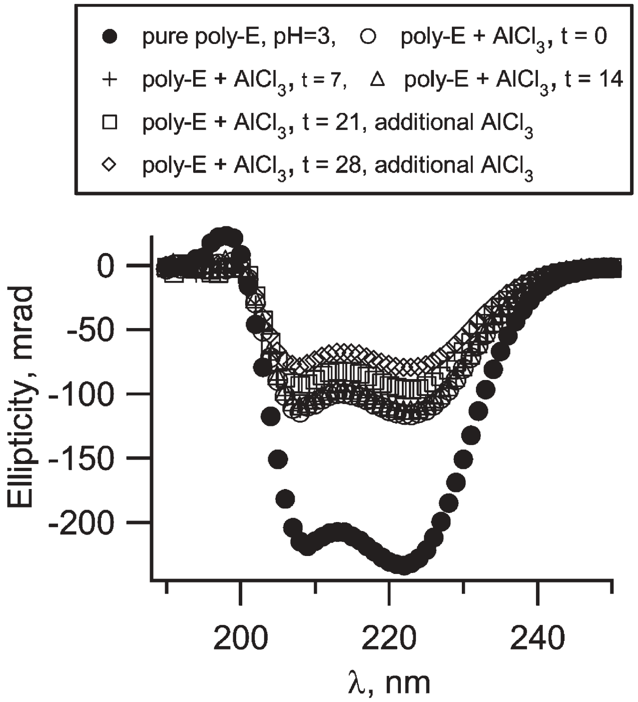

Fig. 2 *In situ* circular dichroism measurements of PLGA + aluminium chloride solutions. Times given in figure are in minutes.

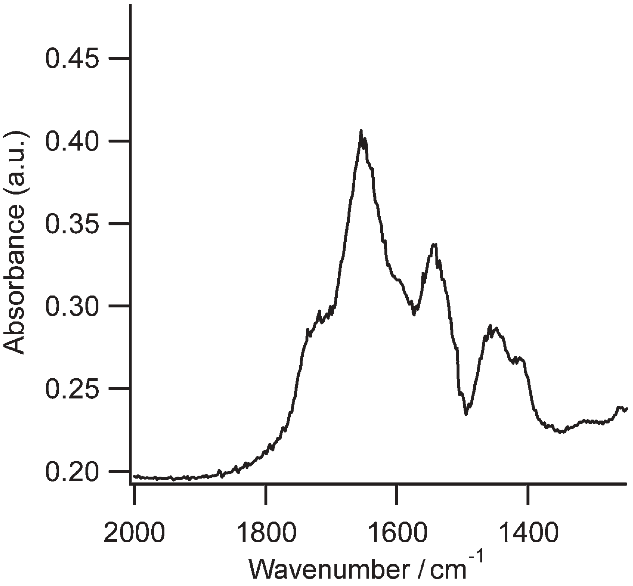

Fig. 3 IR spectrum for alumina–PLGA composite material.

In addition to the aluminas shown above, we also explored the synthesis of porous iron oxides in the presence of poly-L-glutamic acid. The interpretation of these experiments is much more ambiguous than the results shown above for the aluminas. The supporting information contains the adsorption data, CD, and IR results for the iron oxide samples.† These materials, analogous to the aluminas, are amorphous. Given the lack of suitable reference iron oxide materials for use in α_s-analyses, making quantitative conclusions about pore size distributions and micropore/mesopore volumes based on adsorption data is not warranted.[14] The materials have large surface areas by BET analysis (200 $m^2 g^{-1}$) but appear to possess low porosity. However this is inconsistent with the fact that it is very difficult to remove the PLGA from the composite material, and that in fact the sample still contains PLGA by IR *after two extractions overnight in refluxing ethanol*. The FE-SEM shows the particles are small (<50 nm) but TEM indicates they are porous. CD analysis and IR are also less conclusive, as the IR indicates that both protonated and deprotonated PLGA are occluded in the as-made composite material. Given the iron phase formed is likely an iron hydroxyoxide, the presence of deprotonated side chains groups may not indicate an unfolded state as compared to what is usually observed in solution. While the details of the iron hydroxy oxide systems are less clear than the aluminas, they are unusual and potentially very interesting materials.

In summary, here we show that poly-L-glutamic acid can be used to synthesize porous aluminium oxide. These materials, which do not possess any long range order, have appreciable porosity. *In situ* CD measurements indicate that the PLGA in solution remains folded during synthesis. IR measurements of the composite materials show that in the alumina the PLGA retains its helical conformation. The results as a whole show that PLGA has a clear templating effect in the aluminas while the results are more ambiguous in the case of the iron materials. However in both cases unique materials are formed. This work expands on our previous work in the silica–poly-L-lysine system, indicating that using folded helical polypeptide as templates for fabricating porous oxides is a concept that appears to be generally applicable.

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Jeng-Shiung Jan and Daniel F. Shantz*
Department of Chemical Engineering, Texas A&M University, TAMU 3122, College Station, TX 77843-3122, U. S. A.
E-mail: shantz@che.tamu.edu; Fax: +1 979-845-6446;
Tel: +1 979-845-3492

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