Effects of Nanoscale Pore Size and Microscale Particle Size on Diffusion of Proteinase K out of Sol-Gel Silica

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Statement of Purpose: Silica particles derived from the sol-gel method is an inorganic material that is uniquely suited to applications in biology. Not only is amorphous silica biologically inert and biocompatible, the sol-gel synthesis is extremely versatile. Encapsulation of numerous biomolecules in sol-gel derived silica has been shown to be possible.[1] In most cases, the biomolecules are observed to be more stable than in solution. Additionally, the nature of the sol-gel synthesis provides that the particle size, density, and pore size of the silica particles can be easily controlled. The aim of this project is to study the effects of microscale particle size and nanoscale pore size of sol-gel derived silica on Proteinase K release rate. The effects of nanoscale porosity on the stability of the enzyme was also studied.

Methods: (materials and analytical procedures used) Silica particles were synthesized through the sol-gel method (through the hydrolysis and condensation of TEOS, tetraethylorthosilicate). By controlling the ratio of the reactants, the transition from a liquid (sol) to solid silica network (gel) was sufficiently long (20 minutes) to introduce and entrap the enzyme into the solid silica network. Proteinase K was introduced to the sol at 26°C and pH 6. Microparticles of various sizes (250 um – 20 um) were synthesized through grinding and the emulsion method. The porosity and pore size of the particles were controlled through the particle drying technique employed. Supercritical drying resulted in silica aerogels (~90% porous with pores in the range of 20 nm across) solvent exchange resulted in ambigels (~80% porous with 50 nm pore diameters), and ambient evaporation of reaction byproducts produced xerogels (~65% porous with pores larger than 100 nm). The particles were then immersed in buffer to study the mode of release and the diffusion rate of proteinase K out of the silica particles. The activity of the enzymes were measured via fluorescence spectroscopy using EnzChek Protease Assay Kit (Molecular probes), a fluorescence based protease assay.

Results / Discussion:

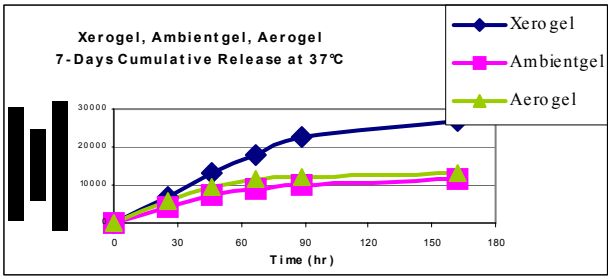

Figure 1: Release rate comparison of sol-gel materials.

Figure 1 shows that xerogels (with lowest overall porosity and largest pores) has not only the highest amount of enzyme released, but only the fastest release rate compared to aerogels and ambigels. This is most likely due to the larger pores in xerogels allowing for easier diffusion. Theoretical single-pore diffusion coefficients were calculated: D(xerogel) = 9.66E-11 m^2/s, D(ambigel) = 7.67E-11 m^2/s, and D(aerogel) = 3.41E-11 m^2/s. Similar studies also showed that the enzyme release rate was inversely proportional to the particle size. Particles with diameters on the order of 60 um showed a 20% increase in enzyme released compared to particles with diameters on the order of 250 um. Microspheres made through the emulsion process also showed better retention of the enzyme, 33% retained compared to that of the ground samples, 19% retained. The release rate is also correlated with temperature (Fig. 2).

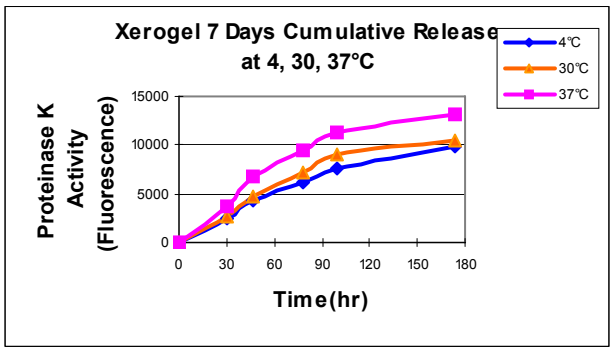

Figure 2: Release rate is dependent on incubation temperature.

Conclusions:
Silica xerogels, with the lowest overall porosity and largest pores showed the highest retention, highest amount of enzyme released, and the fastest release rates compared to silica aerogels and ambigels. The enzyme release rate is inversely proportional to particle size and directly proportional to incubation temperature. The emulsion method also showed better retention of enzyme compared to the ground samples. Recent data (not yet finalized) suggests that the microstructure of xerogels do a better job at stabilizing the enzyme than ambigels and aerogels.

Reference:
[1] Kortesuo, P., Biomaterials, 2000; 21: 193-198.