효소의 encapsulation을 위한 솔-젤 비드의 제작

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Sol-gel bead preparation for enzyme encapsulation

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Introduction

As widely known before, immobilized enzymes have lots of advantages such as multiple usage, easy separation and improved stability. There are several immobilizing techniques, including covalent immobilization, encapsulation and adsorption. Among these methods, we specifically studied encapsulation method using sol-gel technique in this research. Sol-gel methods is biologically inert and can be processed under room temperature and pressure, and the pore size of the matrices can be controled by changing sol conditions. And also its wide-range precursors make it possible be fabricated in many applications. So far, enzyme encapsulated sol-gel matrices for biocatalyst are used as a crushed powder form after xero-gel state. However, to be dried xero-gel state could cause the disruption of the pore and its 3-dimensional structures and moreover, the sol-gel particles followed by crushing process necessarily have irregular shapes and sizes. So in this study I focused on the process to develop the method to make the sol-gel particles in uniform-shaped and sized forms.

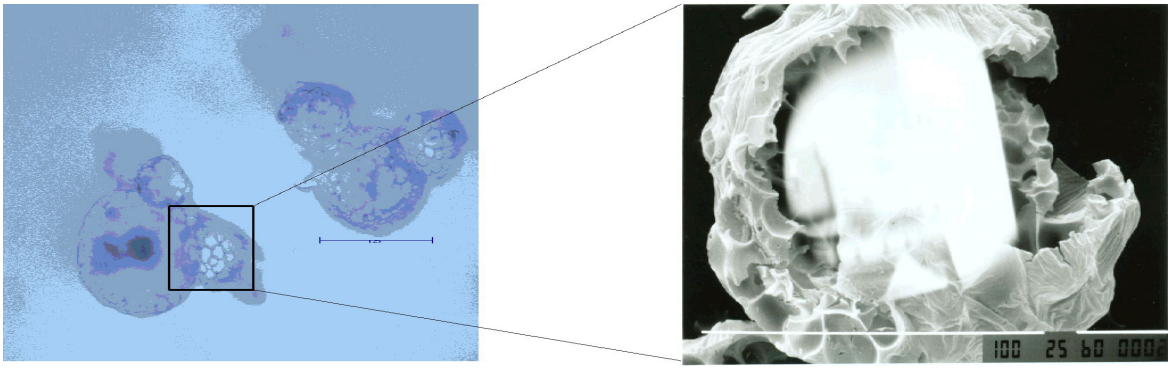
Data and Discussion

Before choosing the proper solvents for sol-gel bead preparation, we tested several precursors such as TMOS(Tetramethoxysilane), TEOS(Tetraethoxysilane) and MTMS (Methyltrimethoxysilane) in various polymerization conditions. Because the polymerization process occurs both acidic and basic environments, this conditions can change the property of the prepared sol-gel beads. After dozens of pre-test, we found that TMOS and MTMS with 1/1 volume ratio(1mL) mixed in pH 7.5 buffer(2mL) as a polymerization condition is the most opportune point.

To make a sol-gel bead a sphere and uniform structure, we focused on the immisciblity between hydrophobic and hydrophilic phases. The most important properties for selecting desirable solvents are polarity and viscosity. We investigated to find proper viscosity at the immiscible range. After testing several solvents in order of the viscosity value, we found out the solvent, cyclohexanone which has moderate viscosity value rho, around 2. This solvent shown most favorable results to make a sol-gel bead regularly. If the viscosity is too low, the sol dispersed in the solvents do not form a sphere shape because two distinctive phases are formed even at high mixing rate. On the other hand, if the viscosity is too high, the solvents are hardly mixed so that the formed particles have irregular and absurd shapes.

After determining the proper solvents, we could get the bead whose size is around 100um with relatively regular sphere shapes. The SEM pictures of the sol-gel particles are not shown

here.

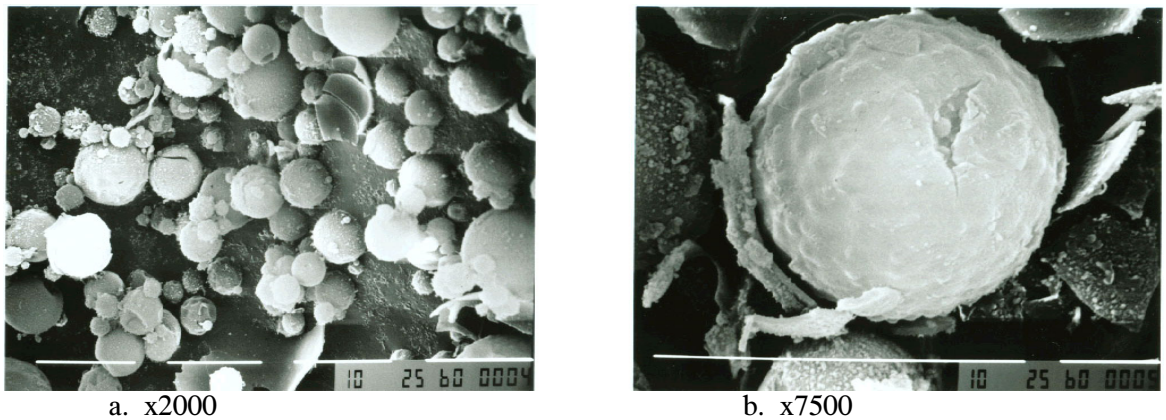


a. Microscopic Picture(x200) b. SEM Picture(x750)

Figure 1. Microscopic and SEM pictures of sol-gel bead prepared with only solvents. The scale bar at the picture b is 100um

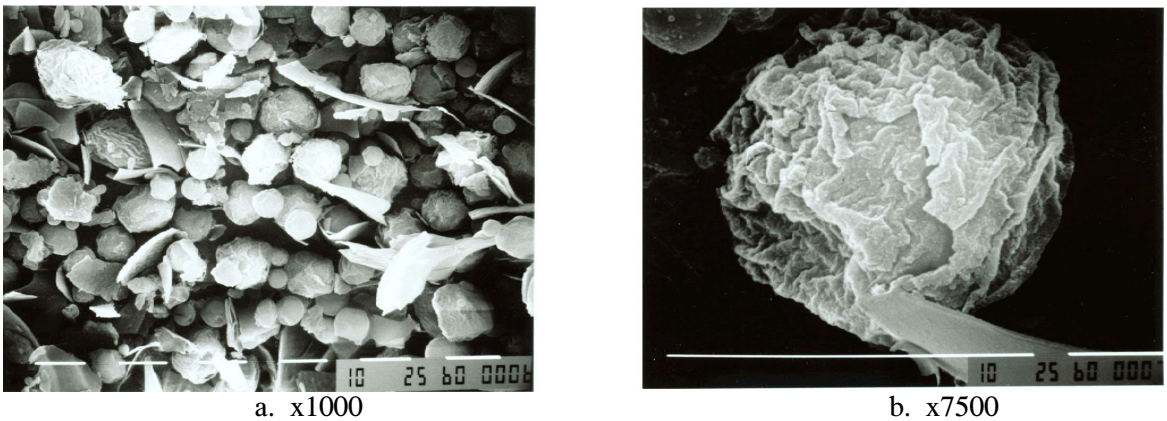
Many macropores are found on the inner structure of the particles. The operational stability of omega transaminase(w-TA), the model enzyme mainly used for testing the sol-gel bead matrices, was relatively good. After 6 repetitive uses, the final conversion value is remained 92% of its initial value. This result strongly imply that enzymes may exist inside the wall not in the pore. If enzymes are inside the macropores, sol-gel beads would lose their enzymatic activity largely after 1st run.

In order to control the size of the bead particle, we added surfactants into the solvents. We tested several kinds of surfactant such as Triton-X series, Triton SP series, Tween 20, and CTAB(Hexadecyltrimethylammonium bromide). All the surfactants are used in 10% volume ratio except CTAB which is used in 10% weight ratio. Triton-X 100 and Triton-X 114 shown successful results in the point of small bead preparation. Their SEM pictures are shown in Figure 2 and 3.



a. x2000 b. x7500

Figure 2. Prepared beads with Triton-X 100. The scale bar in both figures represents 10um a. x2000, b. x7500



a. x1000 b. x7500

Figure 3. Prepared beads with Triton-X 114. The scale bar in both figures represents 10um a. x1000, b. x7500

The figures above shown that the particle size, shape and its distribution become more regular after using surfactants. But the enzymatic activity encapsulated in the matrices are quite different. The operational stabilities of the beads mentioned above are shown in Figure 4.

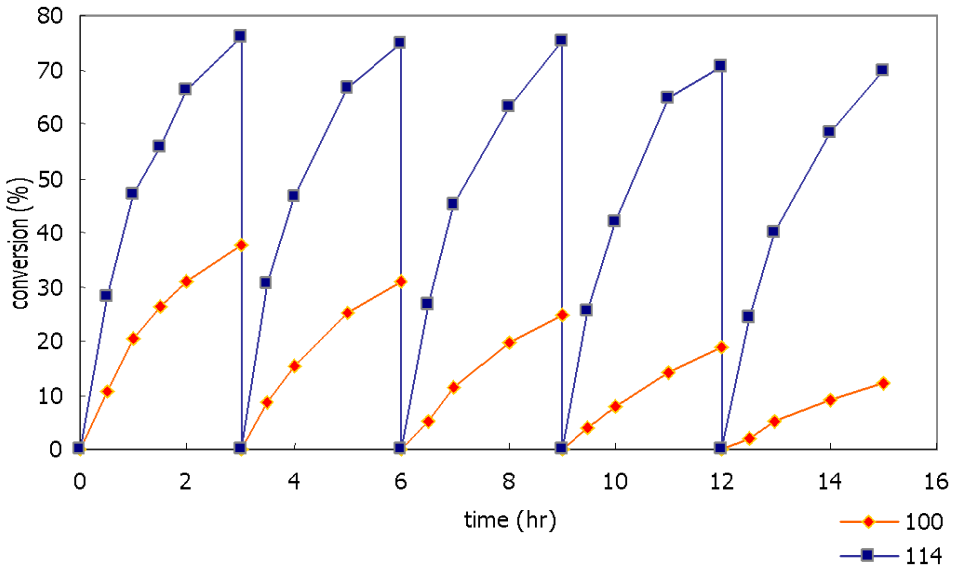


Figure 4. The operational stabilities of the beads prepared with Triton-X 100 and Triton-X 114.

Beads with Triton-X 114 maintains its activity up to 92% after 5 uses while beads with Triton-X 100 has only 34% of its initial conversion rate. This means the significant amounts of enzymes encapsulated into the sol-gel beads prepared with Triton-X 100 were leak after every reactions.

Also Triton SP 135 and Triton SP 190 shown good results in their enzymatic activities of encapsulated w-TA. We took a SEM pictures of these 2 examples but, because of the blunder during drying process, we could not get a fine SEM pictures in this time. So we have only operational stability data for Triton SP 135 and 190 samples. They are shown in Figure 5.

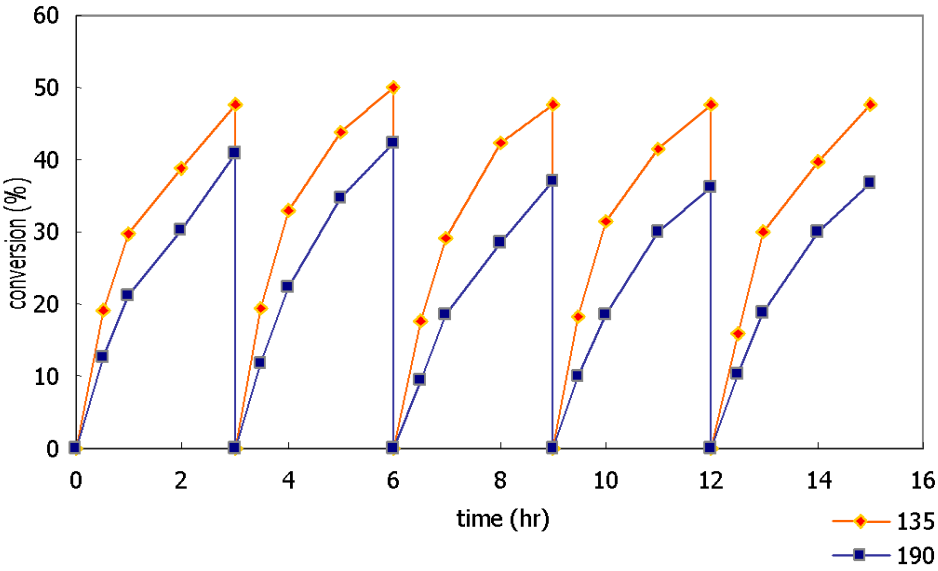


Figure 5. Operational stabilities of the sol-gel beads prepared with Triton SP 135 and 190

The encapsulated enzymes prepared with Triton SP 135 has the best operational stability for w-TA. It maintains 99% of its initial conversion rate after 5 uses. In this experiment, the encapsulated enzymes into the beads prepared with Triton SP 190 shows 90% after 5 uses.

To test the availability of other enzymes, we used lipase to encapsulate into the sol-gel beads. In this experiment, we just find out the enzyme's relative activity to that of free enzyme and Triton-X 114 and Triton SP 190 were used as surfactants. The lipases encapsulated into beads with Triton-X 114 shows 21% and that of Triton SP 190 was just 13%.

Conclusion

We successfully prepare the sol-gel beads with uniform shape and relatively small size. Also the encapsulated enzymes activity in terms of operational stability and relative activity to the free enzyme are favorable. Triton-X 114, Triton SP 190 and Triton SP 135 are selected from many surfactant candidates in that the enzyme's activity as well as the shape and size of prepared beads. The size of the prepared beads is around 10um and the shape of the beads is uniform sphere form. The operational stabilities in the term of the ratio between the initial conversion rate and final conversion rate after 5 uses of the encapsulated enzymes are over 91% up to 99% in the case of Triton SP 135. Also this system works not only for the w-TA but also lipase. Because this is an ongoing work, several works remain to be performed such as size distribution analysis and availability of other enzymes.