# 유기인산 화합물 검출을 위한 poly(ethylene glycol) 마이크로입자 내 효소-형광물질 결합체의 캡슐화

이영식, 최동길[1], 고원건[1], 김범상*
홍익대학교 화학공학과
[1]연세대학교 화학공학과
(bskim@hongik.ac.kr*)

# Encapsulation of enzyme –fluorophore conjugates within poly(ethylene glycol) microparticles for detection of organophosphorus compounds

Youngsik Lee, Dongkil Choi[1], Won-Gun Koh[1], Bumsang Kim*
Department of Chemical Engineering, Hongik University
[1]Department of Chemical Engineering, Yonsei University
(bskim@hongik.ac.kr*)

## Introduction

Miniaturization of analytical devices in micro total analytical systems (μ-TAS) represents a natural extension of microfabrication technology to chemistry and biology with applications in high throughput screening and in portable analytical measurement devices. The recent developments in μ-TAS have allowed the miniaturization and integration of biosensors into a lab-on-a-chip. The μ-TAS creates various advantages over benchtop equipments, including smaller sample consumption, shorter analysis time, low cost, greater sensitivity, low energy and the capability performing on-site analysis. Optical biosensors using immobilized enzymes and proteins are important for monitoring toxic components in the environmental field. Anticholinesterase agents such as organophosphates and carbamate are extremely toxic chemicals and commonly found in a large number of agricultural pesticides as well as chemical warfare agents such as sarin, soman, and cyclosarin. It is well known that these compounds exert their biological effects by inhibition of the enzyme acetylcholinesterase (AChE). The inhibition of AChE increases the amount of acetylcholine (ACh) at central and peripheral sites of the nerve system. High doses of anticholinesterase agents cause convulsions and paralysis of the respiratory muscles. In this study, we prepared and characterized poly(ethylene glycol) (PEG) microparticles containing the conjugate of AChE and pH-sensitive fluorophore (SNAFL-1) to develop microanalysis devices for the detection of organophosphorus compounds. The conjugate of AChE-

SNAFL were encapsulated in PEG microparticles by suspension photopolymerization and the properties of particles as a biosensor was investigated.

## Experimental

AChE-SNAFL conjugate-containing PEG microparticles were synthesized via suspension photopolymerization of an aqueous monomer mixture in a continuous phase of silicone oil. Before the photopolymerization, 0.48 mg of AChE and 1 mg of SNAFL-1 were dissolved in 1 mL of deionized water. EDC and NHS were prepared by solution in phosphate buffered saline (PBS, pH 7.4). The solution of AChE and SNAFL-1 were reacted with the coupling agents which were EDC/NHS for 24 hours at room temperature. The monomer mixture was prepared by mixing 0.87 g of poly(ethylene glycol) methacrylate (PEGMA), 0.018 g of poly(ethylene glycol) dimethacrylate (PEGDMA) as a cross-linking agent, 0.22 g of deionized water, 0.018 g of Irgacure® 184 as a UV initiator, 0.26 g of Span® 20 as a surfactant, and 2.5 mL of AChE-SNAFL conjugate solution. The monomer mixture was then added to the 50 mL of silicone oil and the mixture of oil. The monomer mixture were purged with nitrogen gas for 5 minutes and stirred at 10,000 rpm for 10 minutes at room temperature to form the suspension. For the polymerization the suspension solution was irradiated with UV light for 300 seconds. The synthesized particles were separated from the oil by repeated dilution with deionized water and centrifugation at least five times. The washed microparticles were dispersed with 50mL PBS. The fluorescent properties of the AChE-SNAFL conjugate-containing PEG microparticles were investigated using a fluorescent microscope and a fluorescent spectrophotometer.

## Results and discussion

To verify that the AChE-SNAFL conjugates were encapsulated in PEG microparticles, the particles were observed using a fluorescent microscope. Fig. 1 represents the fluorescent microscope image of the synthesized AChE-SNAFL conjugate-containing microparticles. The particles in the image were shown in green color when the filter with excitation 460~490 nm and emission 520 nm was used. This result indicated that the conjugate of AChE and SNAFL-l were evenly encapsulated in the particles. The pH-sensitive dye of SNFL-1 has an acidic emission peak of 545nm and a basic emission peak of 625nm which allow the pH sensitivity of the fluorophore to be correlated to the ratio of the fluorescent intensities at the two peaks according to the environmental pH changes. The pH-sensitivity of the SNFL-1 after conjugation and encapsulation was examined by changing the pH of the particle solution. As shown in Fig. 2, the emission intensity ratio of the particle changed linearly with pH between pH 4 and pH 10. The emission intensity ratio decreased when the pH increased since the acidic emission peak at 545 nm decreased while the basic emission peak at 625 nm increased in a basic environment.

The enzyme-catalyzed reaction in the particles in response to the amount of the substrate acetylcholinechloride (AChCl) causes a change in the production of acetic acid leading to the microenvironmental pH change. Therefore, the concentration of AChCl could be determined by calculating the ratio of the emission peaks of SNAFL-1 at 545 nm to at 625 nm. Fig. 3 shows the change of the emission intensity ratio of the AChE and SNAFL-1 mixture as a function of the amount of AChCl before they were conjugated. When the concentration of AChCl increased the emission intensity ratio increased resulting from the increase of acetic acid production. The result demonstrated that this system could correlate the analyte concentration to the fluorescent intensity ratio. Finally, the fluorescent behavior of enzyme and fluorophore after conjugation and encapsulation was investigated. The emission intensity ratio as a function of AChCl concentration is shown in Fig. 4. As expected, the change in emission intensity ratio increased with substrate concentration because microenvironment pH in PEG microparticles decreased by the production of acetic acid due to the enzyme reaction.

## Conclusions

The AChE-SNAFL conjugates were successfully encapsulated into PEG microparticles via suspension polymerization. The activities of the enzyme and fluorophore were maintained after they were conjugated and the conjugates were encapsulated in the PEG particles. The fluorescent behavior of the AChE-SNAFL conjugate-containing PEG microparticles resulting form the enzyme reaction between AChE and AChCl indicated that this system could be used for the μ-TAS to detect organophosphate compounds.

## References

[1] Vamsi K. Yadavalli, Won-Gun Koh, George J. Lazur, Michael V. Pishko, "Microfabricated protein–containing poly(ethylene glycol) hydrogel arrays for biosensing", J. Sensors and Actuators B., **97**, 290-297 (2004).

[2] Se-Hwa Kim, Bumsang Kim, Vamsi K. Yadavalli, Michael V. Pishko, "Encapsulation of Enzymes within Polymer Spheres To Create Optical Nanosensors for Oxidative Stress", J. Analytical Chemistry., **77**, 6828-6830 (2005).

[3] Won-Gun Koh, Michael Pishko, "Immobilization of multi-enzyme microreactors inside microfluidic devices", J. Sensors and Actuators B., **106**, 336-338 (2005).

[4] Huiguang Zhu, Rohit Srivastava, J. Quincy Brown, Michael J.McShane, "Combined Physical and Chemical Immobilization of Glucose Oxidase in Alginate Microspheres Improves Stability of Encapsulation and Activity", J. Bioconjugate Chem., **16**, 1451-1458 (2005).

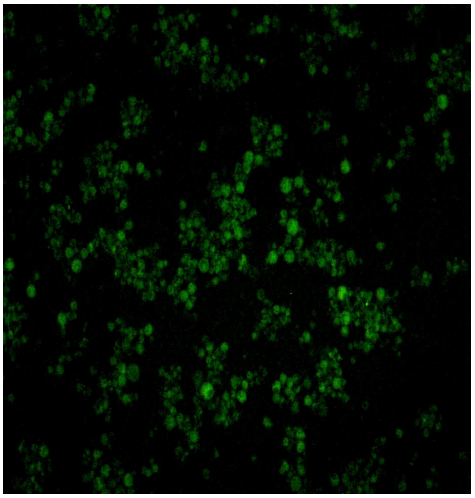

**Fig. 1.** Fluorescent microscope image of PEG microparticles containing AChE-SNAFL conjugates (filter with excitation 460~490 nm and emission 520 nm).

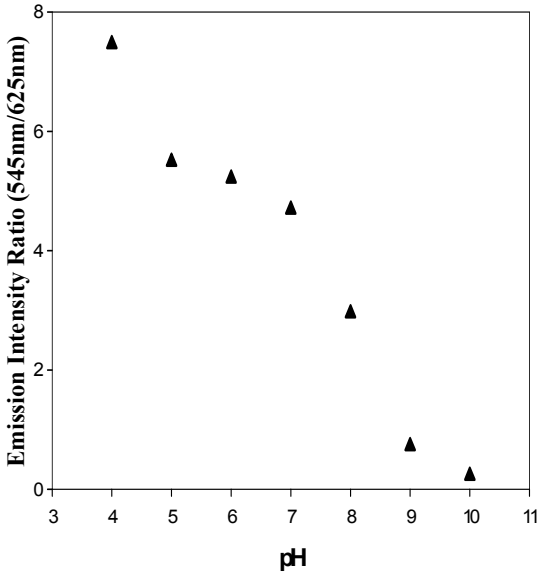


**Fig. 2.** pH-Sensitivity of AChE- SNAFL containing PEG microparticles after encapsulation as a function of pH.

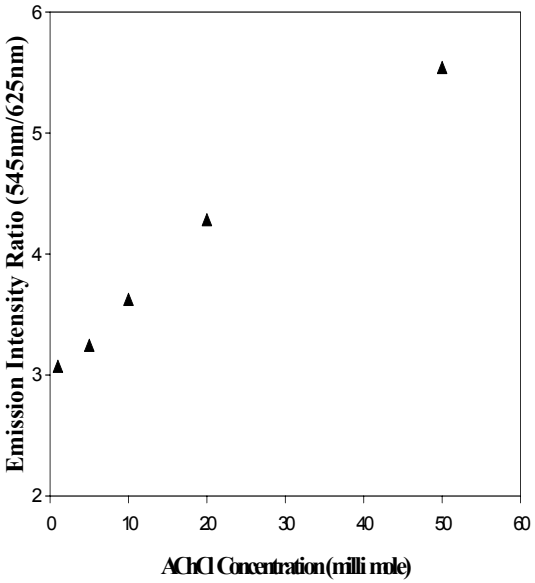


**Fig. 3.** Fluorescence characterization of AChE and SNAFL-1 solution before conjugation; fluorescence emission intensity ratio upon addition of acetylcholinechloride (AChCl).

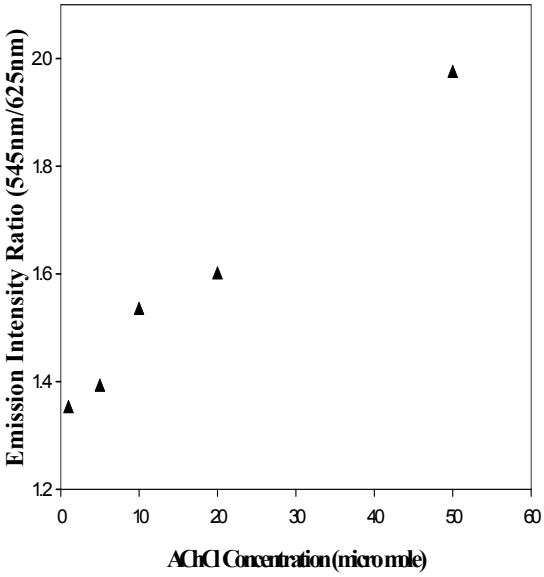


**Fig. 4.** Fluorescence characterization of AChE- SNAFL containing PEG microparticles after encapsulation; fluorescence emission intensity ratio upon addition of acetylcholinechloride (AChCl).