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## Imaging the Distribution and Secondary Structure of Enzymes on Different Matrixes Using Infrared Microspectroscopy

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**Introduction:** Enzymes catalyze a plethora of reactions with high specificity, speed, and yield. However, in order to be useful as industrial biocatalysts, improvements in enzyme stability, activity, and recovery are necessary. Traditionally, these obstacles have been addressed by the immobilization of enzymes on polymer or ceramic matrices. A critical parameter in the performance of an immobilized enzyme is the spatial distribution of the enzyme and substrate within a macroporous resin. [1]

**Methods and Materials:** Lipase B immobilized on different matrixes were gifts from Novozymes (Denmark). The experimental detail for sample embedding, synchrotron IR microspectroscopy and data analysis were described in reference 2.

**Results:** Recently, We developed a nondestructive method to determine the enzyme distribution and secondary structure by Synchrotron IR Microspectroscopy. [2] The experimental detail was provided in the experimental section. Briefly, the enzyme beads were sectioned into 10  $\mu\text{m}$  slides and IR spectra were recorded by a grid-like pattern automatically. The protein distribution was calculated by plotting the amide I bands. Accurel and QDE 2-3-4 are irregular and the other resins are regular circular and Amberlite 1180 and Amberlite XAD7HP had core-shell structure. Interestingly, all the enzymes were shell-like distributed except QDE 2-3-4. The shell thickness of different matrixes was listed in Table 1. Enzyme immobilized on QDE 2-3-4 has shown the highest catalytic activity for enzyme-catalyzed polymerization and enzyme seems randomly distributed on QDE 2-3-4. This is very interestingly because enzymes were reported shell-like distributed in most cases and the study on the effect of random distributed enzyme on the catalytic activity was needed. The secondary structure of the enzyme immobilized on different matrixes were compared with each other. It's found that immobilization of CALB on the different matrixes did not cause a change in its conformation to an extent that can be detected by IR analysis.

Table 1. The shell thickness of enzyme on the different matrixes

	Accurl	Amberlite 1180	Amberlite XAD7HP	Deloxane	Novozyme435	Purolite
Thickness ( $\mu\text{m}$ )	0*	60	50	0*	100	0*

0\*: the enzymes penetrate the support matrix throughout.

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### References:

- [1]. Aires, R. "The mathematical theory of diffusion and reaction in permeable catalysts", Clarendon Press, Oxford, 1975.
- [2]. Y. Mei, L. Miller, W. Gao, R. Gross, "Imaging the Distribution and Secondary Structure of Immobilized Enzymes Using Infrared Microspectroscopy", *Biomacromolecules*, in press.