# Analysis of Temperature Dependence and Effective Collisions for the Eutectic Mixture Formations of Amino Acids Substrates

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## Abstract

The eutectic mixture is a homogeneous solution which consists of more than two hydrophobic compounds with a melting point lower than the melting points of each pure compound[1,2]. In recent years, enzymatic reactions using eutectic substrate mixtures have been developed for solvent-free and high substrate concentrations of biocatalysis[3~5]. In this study, we investigated the temperature dependence and collision properties of a eutectic mixture. A mixture of *N*-CBZ-L-Asp and L-PheOMe·HCl was used as a substrate model, and eutectic formations were performed in a glass water jacket with the adjuvant methanol. We preliminarily obtained a phase diagram of the substrate mixture using a DSC. When the mole fraction of *N*-CBZ-L-Asp increased from 0.3 to 0.8, the melting rate increased until 0.55, but decreased after that. The maximum eutectic-forming rate was obtained at a *N*-CBZ-L-Asp mole fraction of 0.55. An Arrhenius plot of rate constants was used to detect the temperature dependence of eutectic mixture. As the mole fraction of *N*-CBZ-L-Asp increased, the activation energy($E_a$) and pre-exponential factor(A) increased. The highest values of 144.8 kJ/mol and $3.5\times10^{20}$ $s^{-1}$ were attained at a *N*-CBZ-L-Asp mole fraction of 0.55. When the mole fraction was elevated above 0.55, $E_a$ and A were rapidly diminished. Similar activation energies were obtained at mole fractions of 0.4 and 0.8, but the pre-exponential factor at 0.8 were ten times higher than that at 0.4. The highest values of $E_a$ and A were obtained when methanol was added at 32% as an adjuvant at a constant *N*-CBZ-L-Asp mole fraction of 0.55. The numbers of effective collisions were simply adjusted by multiplying all the molecule numbers for eutectic mixture formation. They were clearly matched with the profiles of $E_a$, A, and rate constants.

Especially, the maximum effective collisions were obtained at a *N*-CBZ-L-Asp mole fraction of 0.53 with 213.4 kJ/mol and $2.4\times10^{31}$ $s^{-1}$, respectively, for $E_a$ and A.

## References


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