# REVERSE MICELLAR ENZYMOLOGY. LIPASE CATALYZED HYDROLYSIS OF TRIGLYCERIDES AND SYNTHESIS OF SPECIFIC ESTERS

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Micellar enzymology is a new physichochemical approach to biochemical research problems. These model studies of enzymic catalysis in microheterogeneous media are of great importance in understanding of the enzyme functioning in natural lipid systems. In such studies, the free enzymes are not handled in classical aqueous buffers, but in reverse micellar systems of water in organic solvents (microemulsions) (1).

Water-in-oil microemulsions, or reverse micellar systems, are thermodynamically stable and optically isotropic solutions, with water constituting the dispersed phase, separated from the continuous organic phase by surfactant molecules (2). The enzyme molecules can be encapsulated in the reverse micelles, avoiding direct contact with the organic solvent, that may cause denaturation (3). The despersed water-pools act like a microreactor, with a favorable aqueous microenvironment for enzyme activity, and an enormous interface, through which lipophilic substrates can be cleaved (4,5).

Lipase is the enzyme which catalyzes the hydrolysis of fats to glycerol and free acids. The reaction is reversible and the enzyme can be shown to catalyze the synthesis and the transesterification of triglycerides. This catalytic process of great biotechnological interest, is heterogenous and may be favoured by the use of microemulsions, since the interface through which the reaction occurs, is considerably increased.

Our study deals with the catalytic behavior of lipase from *Rhizopus delemar* in different types of microemulsion systems.

In the case of hydrolysis studies, three different types of

microemulsions were tested, by using anionic (AOT), cationic (CTAB) and ninionic ($C_{12}EO_4$) surfactants. Various parameters affecting the reaction, such as temperature, pH optimum, water content, as well as $K_{m,app}$ and $V_{app}$, were determined using triolein and tributyrin as substrates.

In the anionic AOT/isooctane systems maximun enzyme activity was obtained at pH = 6.45, T = 30°C and R = 9, where 9 expresses the hydration ratio $(H_2O)/(surfactant)$. For triolein the $K_{m,app}$ value is $4.67 \times 10^{-2}$M and for tributyrin $6,96 \times 10^{-2}$M, as calculated from the Lineweaver-Burk double reciprocal plots. The apparent V for triolein was found 143 μmol $min^{-1}mg^{-1}$, while for tributyrin it was found 123 μmol $min^{-1}mg^{-1}$.

In the case of the cationic CTAB/Decane/Hexanol systems, the optimun conditions were found at R = 7, T = 22.5°C and pH = 5.8. The apparent $K_m$ and V values were found 0.10M and 36 μmol $min^{-1}mg^{-1}$ for triolein and 47 μmol $min^{-1}mg^{-1}$ and 0.32M for tributyrin, respectively.

The stability of the enzyme was also studied in anionic and cationic systems, showing a more stable behavior in the latter case. The enzymic reaction of hydrolysis was found to be very slow when it was studied in the $C_{12}EO_4$/Decane nonionic systems.

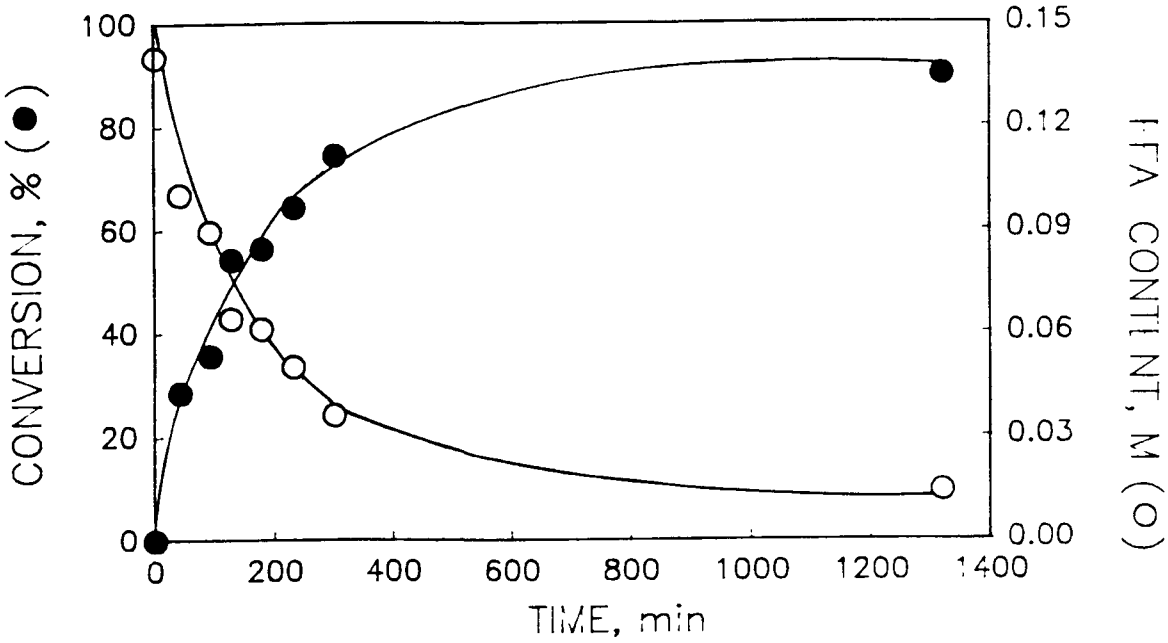


***Fig. 1.*** *Oleic acid esterification by hexanol. (A) Hexyl oleate synthesis expressed as %of conversion (o). Free fatty acid content (M) (o). Detection by GC. Enzyme concentration 120 U/ml. Total microemulsion volume 1ml. T = 30°C.*

In another set of experiments, the esterification of an aliphatic alcohol (hexanol) and a natural fatty acid, such as oleic, myristic or butyric acid, was studied. The reaction was carried out in similar nonionic microemulsions and it was followed by TLC and GC. Fig. 1 shows the reaction profile as determined by the appearance of product vs. time. It can be noticed that after 5 hours of reaction only traces of free oleic acid were observed, when 120 U/ml of enzyme were used. All products were identified by NMR and IR spectroscopy. It is interesting to note that the reaction yield is considerably higher than in similar studies performed in other heterogeneous media. Namely, a yield of only 70% is reported for the synthesis of heptyl-oleate, after a reaction time of 6 days (6).

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