LECITHIN BASED w/o MICROEMULSION SYSTEMS. A NON TOXICAL MICROENVIRONMENT FOR ENZYME STUDIES.

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Microemulsions are optically isotropic and thermodynamically stable solutions of water,oil (hydrophobic/non-polar solvent) and surfactants. Surfactants are molecules with hydrophobic and hydrophilic moieties in their molecule. These amphiphiles are localized at the interface (water-oil) and oriented in such a way to expose the above different moieties (polar and non polar) to the different solvents (1). The water in oil (w/o) microemulsions are fine dispersions of water in a non polar solvent. The droplets of the water phase are surrounded by surfactant molecules, forming the so-called reverse micelles.

It is well established that many enzymes can retain their catalytic ability when hosted in the aqueous core of the microemulsions (2). These enzyme-containing reverse micelles can be considered as microreactors able to transform substances with different polar character. So far, most of the studies involving enzymic systems have been performed by using well characterized artificial surfactants (3). Nevertheless, these molecules are generally toxic and cannot be used in potential pharmaceutical applications of enzyme containing microemulsions (4). An attractive alternative could be the use as surfactants of natural emulsifiers such as lecithin.

In this work we present the results of trypsin proteolytic activity studies carried out in microemulsion systems formulated with soya bean lecithin. The phase diagram presenting the limits of the monophasic area of the system partially purified lecithin/isooctane/water/propanol-1 is shown in Figure 1.

Various parameters affecting the enzyme activity, such as the pH and the water content expressed in terms of the molar ratio $w_o = [H_2O]/[lecithin]$ were examined in micro-emulsions of different compositions. The catalytic ability of trypsin to cleave model substrates, such as L-lysine p-nitroanilide was tested and it was shown to follow Michaelis-Menten kinetics, allowing the determination of the relevant constants K_m and K_{cat}. In all cases the K_{cat} value increases when the catalysis takes place in a microemulsion system as compared to the value obtained in the aqueous solution (Table I)(3)

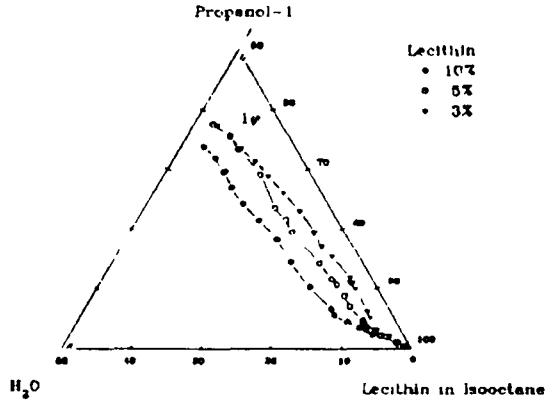

Fig.1 Ternary phase diagram of the system lecithin/isooctane/propanol-1/water

Table I

	K_m (M)	K_{cat} (s^{-1})	K_{cat}/K_m ($M^{-1}s^{-1}$)
microemulsion	0.00498	0.0900	18.20
aqueous solution	0.00052	0.0114	21.90

Microemulsion system: 5% w/w Lecithin/Isooctane/Propanol-1/Water. Lecithin/Isooctane: Propanol-1 9:1 v/v, Water 1.5% v/v (w_o=20.9), T=30°C, pH 8.5 (buffer Tris/HCl 0.1 M)

Furthermore the stability of trypsin in this medium was examined in comparison to the aqueous solution in the same conditions. It was shown that trypsin maintains after 24 h, 25% of its activity in the microemulsion, and only 6% in the aqueous solution.

To investigate the structure of these microreactors, fluorescence quenching experiments have been carried out in the presence and in the absence of the enzyme. For this purpose we have used $Ru(bpy)_3^{+2}$ as the lumophore and $Fe(CN)_6^{-3}$ as the quencher (Fig.2). We have examined the effect of the water content in this phenomenon and the preliminary results show that the system undergoes a percolation procedure when the water content is 3% v/v (w_o=43.4).

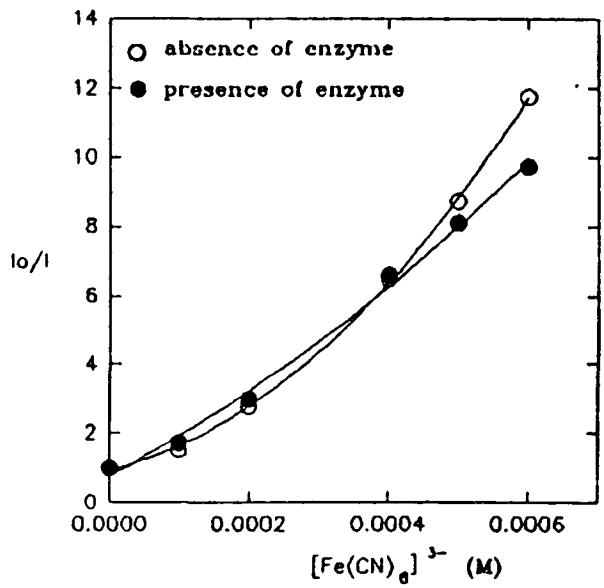

Fig.2 Fluorescence quenching of $Ru(bpy)_3^{+2}$ in microemulsions by various concentrations of $[Fe(CN)_6]^{-3}$ in the presence and in the absence of trypsin

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