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ENZYME-CATALYZED ESTERIFICATIONS IN MICROEMULSION-BASED ORGANO GELS

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Summary: A new alternative for enzyme-catalyzed reactions in organic media is described, involving immobilization of the enzyme in microemulsion-based gels (MBG's). The method is applied to the esterification of oleic acid with several alcohols in various organic solvents.

The importance of enzymatic synthesis in organic solvents is well recognised[1,2], and a number of different methods for carrying out enzymic conversions in these media are found in the literature[3-10]. We now describe a new alternative for enzyme-catalyzed reactions in organic media which eliminates some disadvantages of other methods, such as the use of a large excess of the enzyme[11-13] and the impaired activity of these biocatalysts in organic solvents[10,14].

By the use of immobilized enzymes in microemulsion-based organo-gels (MBG's), low, known concentrations of the catalyst may be immobilized in the water pool of a reverse micelle which is "frozen" in a gel. When dipped into an organic solvent, this insoluble gel is the source of enzymatic catalysis in a convenient two-phase system for reactions taking place in an organic solvent.

The properties and stability of these gels in organic solvents have been described[14-17]. In this communication we illustrate their utility in the esterification of oleic acid with several alcohols in various organic solvents.

The microemulsion-based organo-gels were prepared by mixing sodium bis 2-(ethylhexyl)sulphosuccinate (AOT), an organic solvent, an aqueous solution of known concentration of a lipase and a solution of gelatin in water at 55°C. The mixture was shaken vigorously and allowed to cool at room temperature to give a rigid gel. Typically, a mixture of 0.89 g of AOT in 5.3 ml of n-hexane, 2.5 mg of lipase in 2.4 g of distilled water and 1.4 g of gelatin gave, after vigorous shaking, 10 ml of lipase-containing MBG. The organo-gels were frozen at -15°C, cut into cubes of 1 cm^3 volume, and stored in the freeze. The reactions were performed by simply shaking at 25°C an organic solution of oleic acid and an alcohol, to which the sectioned gels were added. The course of the reaction was followed by thin-layer chromatography. The solution containing the products and the unreacted substrates was decanted off, the solvent was rotary-evaporated and

the ester products were isolated by flash chromatography using Merck 60H silica gel and a hexane/diethyl ether mixture (3:2) as eluent.

The characterisation and purity of the obtained esters were confirmed by thin-layer chromatography and by spectral comparison (IR and ^{1}H-NMR) with known samples prepared by standard acid-catalyzed esterifications of oleic acid.

Blank tests were performed without the enzyme and no product was observed.

The results given in Table 1 show that lipase successfully catalyzes this reaction with short, medium and long aliphatic, cyclic, aromatic and unsaturated alcohols.

Straight chain alcohols gave the corresponding esters in high yields.The comparatively lower yields obtained for cyclic alcohols such as cyclohexanol and benzyl alcohol may indicate a diminished reactivity of more hindered substrates. Although this observation is at variance with the good yields obtained for 3-methyl- and 2-methylbutanol, failure of hindered t-butanol to give any ester probably reflects the steric selectivity of c.v. lipase.

The MBG-immobilized enzyme retained its activity after several runs. Thus, average yields of 70% could be obtained with the same gel through 15 different esterification reactions involving oleic acid and n-pentanol. A batch of c.v. lipase immobilized in MBG retained its activity after being kept in the cold for one year. Use of this catalyst after this period gave the oleic acid esters of n-pentanol, n-butanol and n-decanol in 68%, 63% and 76% yield respectively.

Table 1- Alkyl oleates from oleic acid and alcohols (0.01 mol each). The reactions were performed at 25°C in n-hexane for 10 h, in the presence of 250 μg/ml of c.v. lipase, immobilized in 10 ml of sectioned MBG's

Alcohol	Yield(%)
ethanol	61
n-propanol	64[a]
2-propanol	47[a]
n-butanol	63
2-methylbutanol	82
n-pentanol	88
3-methylbutanol	89[a]
n-hexanol	72[a]
cyclohexanol	61
n-octanol	80[a]
n-decanol	82[a]
n-dodecanol	62[a]
n-tetradecanol	68
n-hexadecanol	75[a]
n-octadecanol	74
benzyl alcohol	52
allyl alcohol	50
t-butanol	0

(a) The same MBG-immobilized lipase was reutilized for each conversion.

Microemulsion-based organo gels may be prepared with various organic components. MBG-immobilized lipases prepared with cyclohexane showed the same activity as the system which employed n-hexane as the organic cosolvent. More interesting is the fact that the organic solvent employed in the two-phase esterification process need not be the same cosolvent which was used in the preparation of the MBG's. The proton NMR spectra of a CCl_4 solution of benzyl alcohol before and after a one-hour contact with the gel prepared with n-hexane were identical. This is an indication that during the reaction there is little or no diffusion of the organic cosolvent from the gel lattice into the organic phase.

The stability of these MBG's in different organic media may be exploited when a substrate is scarcely soluble in hexane. Table 2 gives the yields of formation of n-pentyl oleate in various solvents after 24 h of incubation, employing MBG-immobilized c.v. lipase as a catalyst. The observation that n-octane and aliphatic hydrocarbons in general afford the highest yields of esterification agrees with the findings of Klibanov and Zaks that optimal yields of lipase-catalyzed acylation of alcohols take place in octane [18].

The decrease in the yields as the solvent becomes less hydrophobic may be associated with the greater ability of the more polar milieu to pull water from the enzymes that are encapsulated in the MBG's. This seems to be an important criterium for solvent selection in biocatalytic processes [3,14]. The decrease in activity of the immobilized lipase in the water-miscible 1,4-dioxane is thus not surprising.

Table 2 - Yields of n-pentyl oleate from n-pentanol and oleic acid in various solvents at 25°C. The incubation time was 24 h and the catalyst was c.v. lipase immobilized in a microemulsion-based gel prepared with cyclohexane.

Solvent	Yield (%)
octane	88
isooctane	83
n-heptane	82
cyclohexane	79
n-hexane	78
petroleum ether	77
toluene	70
carbon tetrachloride	63
benzene	45
diethyl ether	42
chloroform	12
1,4-dioxane	7
xylene	7

As in all biocatalytic processes, the nature of the enzyme plays a crucial role in determining the yield of conversion. The yields of formation of n-pentyl oleate after 10 h in n-hexane, employing different lipases as catalysts varied from nearly 70% to zero. Lipases ex Chromobacterium viscosum (c.v.) (68%), Pseudomonas fluorescens (55%) and Microbial (62%) showed nearly the same effectiveness, whereas the lipases ex Candida cylindracea (c.c.) and the Porcine Pancreatic Lipase (PPL) formed no ester under the same conditions.

In conclusion, immobilization of enzymes in microemulsion-based gels (MBG's) constitutes an attractive alternative for the existing methods of enzymic catalysis in organic media. The main advantages of the present method include the economical reutilization of small amounts of the enzyme, conveniently immobilized in an aqueous medium where its solubility and activity are maximum.

The method should be applicable to the building of other functionalities and to the resolution of racemic substrates. The scope and applications of this methodology are being investigated in our laboratories.

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