THE PREPARATION OF MICROEMULSION-BASED GELS OF n-HEXANE OR CYCLOHEXANE FOR ENZYME IMMOBILIZATION

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The diagrams of formation of microemulsion-based gels composed of a surfactant (AOT), water, gelatin and an organic solvent are presented for n-hexane and cyclohexane. The behaviour of these gels as enzyme-immobilizing media is discussed in terms of their stability in various organic solvents.

Keywords: microemulsion-based gels; enzyme immobilization.

INTRODUCTION

The use of enzymes as catalysts for highly specific reactions constitutes an area of growing interest. The drawbacks associated with working with aqueous solutions have been in many instances circumvented by developing methods for enzymatic catalysis in organic media[1-5]. Biocatalysis in apolar solvents is an expanding area of research where one strives for maximum enzyme activity coupled with the use of minimum amounts of a reusable catalyst.

The suggestion that microemulsions might be convenient media for enzymatic reactions[6,7] triggered the investigations on practical alternatives for the originally proposed method. Immobilizing the enzyme in a microemulsion-based gel represented a promising development in this line of research. Thus, lipases immobilized in microemulsion-based organogels (MBG's) catalyzed esterifications in n-hexane, retaining their activity after several runs[8,9] .

In trying to widen the scope of this method, so as to encompass a wider range of reactions, solvents and substrates, we decided to investigate the formation of MBG's from different organic solvents. In order to do so, detailed phase diagrams must be obtained, which vary considerably from solvent to solvent, even when they are functionally similar and possess nearly the same polarity.

We have shown that enzymes immobilized in these systems are convenient biocatalysts for esterifications of oleic acid in different solvents[9]. In the present report we analyse and discuss the diagrams of these four-component systems, prepared from different organic solvents.

EXPERIMENTAL

For the preparation of the microemulsion-based gel, gelatin (Sigma Bloom 300) was employed to immobilize a microemulsion obtained by mixing appropriate proportions of the anionic surfactant Aerosol O-T (Sodium bis-2-ethylhexylsulfosuccinate, AOT from Sigma), water and an organic solvent (n-hexane or cyclohexane, Merck AR grade). The gelatin, Aerosol O-T and the organic solvents were used without any further purification. The components were mixed in stoppered tubes and heated at 55°C to ensure a thorough mixing. The final attained volume of the liquid mixture was always 10 ml. The tubes were then cooled to room temperature with vigorous shaking and the stability, homogeneity and transparency of the resulting system were visually evaluated and recorded. A minimum of 260 mixtures of variable composition were prepared and investigated. The resulting points were mapped so as to obtain the diagrams shown in Figures 1 and 2 for systems containing cyclohexane and n-hexane respectively.

Both diagrams were obtained with a constant surfactant concentration, [AOT] = 0.1 M. Thus the molar ratio $w_o = [H_2O]/[AOT]$ is in fact a direct measure of the amount of water present in the system. The gelatin content in the mixture is expressed as overall percentages in grams per 100 ml.

RESULTS AND DISCUSSION

Just as for the reported isooctane/AOT system[6,10], the diagrams shown in Figures 1 and 2 comprise four different regions.

Region I corresponds to a heterogeneous system where precipitation of solid gelatin occurs, as a result of the insuffi-

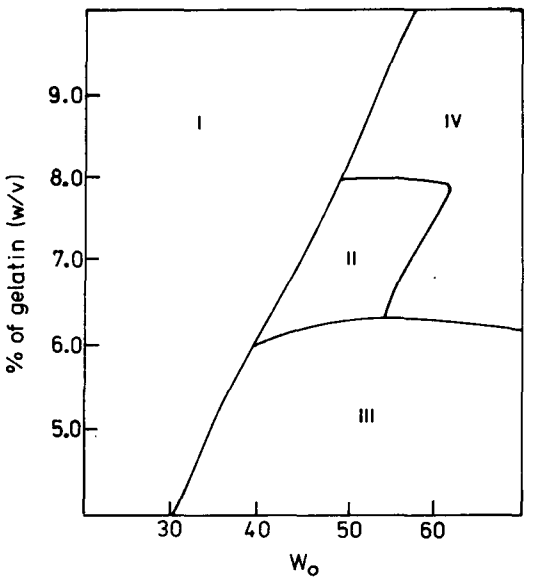

Figure 1. "Phase diagram" of gelatin-water-AOT-cyclohexane for a fixed surfactant concentration of 0.1 M. W_o is the molar ratio $[H_2O]/[AOT]$. The four regions are described as follows: (I) phase separation of solid gelatin; (II) separation of the gel and a liquid phase; (III) micellar liquid solution; (IV) homogeneous solid gel.

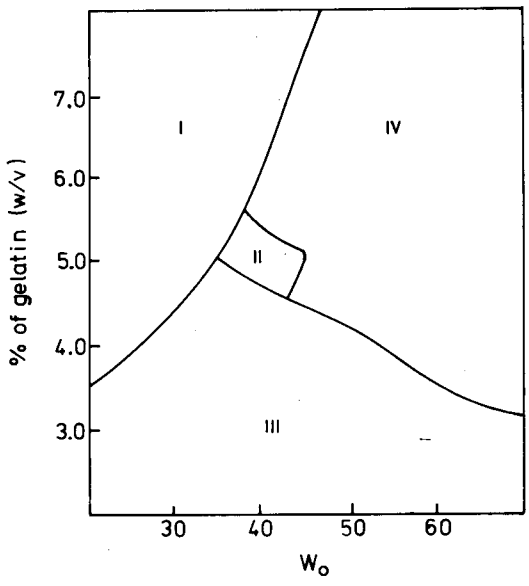

Figure 2. "Phase diagram" of gelatin-water-AOT-n-hexane for [AOT]= 0.1 M. W_o is the molar ratio $[H_2O]/[AOT]$. The four regions are described as follows: (I) phase separation of solid gelatin; (II) separation of the gel and a liquid phase; (III) micellar liquid solution; (IV) homogeneous solid gel.

cient amount of added water which does not solubilize all the gelatin in the mixture. In region II this no longer happens, but phase separation is still observed between the solid gel and a highly viscous liquid. This demixion zone reflects an insufficient amount of gelatin in the mixture, unable to produce a stable, homogeneous gel. A micellar gelatin liquid solution is found in region III. Region IV constitutes the domain of formation of a homogeneous, solid gel. The sol-gel transition from region III to region IV requires the addition of more gelatin to the system.

The ease of formation of a gel for a fixed surfactant concentration is a function both of the water content, expressed by the w_o value, and the amount of added gelatin. It also depends on the apolar solvent present in the system.

Our diagrams agree with previous observations[6,11] that the organogel is not formed at low water contents, where $w_o < 35$. In addition, the apolar solvent is important in determining the upper temperature phase boundary of the microemulsion formed. This temperature, for example, is much lower for heptane than for cyclohexane microemulsions[11]. Accordingly, heptane gels require less gelatin (a minimum of 3.5% w/v) than cyclohexane gels to be formed. This is seen in Figure 1, where the cyclohexane gel is not formed at any w_o value, unless the gelatin content in the mixture is raised to about 6.5%.

The qualitative similarity of the diagrams obtained with cyclohexane and n-hexane is clear from a comparison of Figures 1 and 2. The main difference lies in the sol-gel transition. The concentration of gelatin required for gelation of the liquid system is lower (ca. 4.5%) for the n-hexane than for the cyclohexane gel. In addition, this threshold value is further reduced in the former system upon addition of water to the mixture, attaining a concentration value of nearly 3% gelatin at w_o=70.

The organogels are surprisingly stable in apolar solvents. This observation, which is the basis for the use of these systems in biocatalytic processes in organic media[8,9], could hardly be anticipated on the ground of the high content of the apolar solvent in the gel.

In a more polar medium, however, the gel may be less stable. In fact, when dipped into a polar solvent, the transparent organogel became eventually opaque with time, or was even destroyed. A macroscopic inspection of the gel was carried out to define the solvents to be employed in enzyme-immobilized processes. Solvents such as acetone, dimethylformamide, dioxane, dimethylsulfoxide, or water attacked our gels, whereas less polar solvents such diethyl ether, chloroform, carbon tetrachloride, and hydrocarbons in general were inert. Protic solvents such as acetic, propionic and butyric acids also destroyed the gel. The same was true of ethylene glycol, methanol and ethanol whereas alkanethiols (1-propanethiol, 2-butanethiol and thiophenol) were in general more inert.

The fact that the microemulsion-based gels are less stable in hydrophilic solvents indicates that a minimum amount of water is essential to stabilize the gelatin network of the gel. A solvent which competes with the gelatin for this water will eventually disrupt the gel.

In more hydrophobic solvents however, such as n-hexane or chloroform, microemulsion-based gels are quite effective supports for enzymes.

As an illustration of the use of the systems described above, we carried out the reaction of oleic acid (0.01 mol) with 1-pentanol (0.01 mol) in cyclohexane (30 ml) at 25°C in the presence of 10 ml of MBG's. The esterification was catalyzed by cv lipase (0.25 mg/ml of added water) immobilized in a microemulsion-based gel of hexane ([AOT] = 0.2 M, w_o = 60, gelatin concentration of 1.4g/10 ml of gel), and yielded, after 10 h, 79% of the product. The separated gel was then extracted for 24 h with 4-5 portions (20 ml each) of cyclohexane, until no more ester could be detected in the organic phase. We thus obtained an additional (9%) crop of the product.

Thorough washing of the gel with the apolar solvent after completion of the reaction is thus recommended to ensure better yields and the purity of the products of subsequent preparations, if the gel is reutilized again.

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