

# SURFACTANT AGGREGATION IN HYDROPHOBIC IONIC LIQUID TO FORMULATE MICROEMULSIONS FOR THE ENHANCEMENT OF THE SOLUBILITY OF ENZYMES AND THEIR CATALYTIC PERFORMANCE

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Room temperature ionic liquids (ILs) are molten salts at room temperature or below 100 °C. They are composed of organic cations and inorganic/organic anions. ILs have many advantages such as low volatility, high stability, good miscibility with organic compounds and unique constituents designability. Compared to traditional organic solvents, ILs are usually considered as “green” solvents. The use of ILs as media for enzyme catalysis was tried as early as 2000, and since then, many endeavours have been devoted to the studies.

Previous studies indicate that an enzyme usually has catalytic activity in ILs if the enzyme is active in an organic solvent. Also it has been found that the catalytic performance of enzymes in ILs is correlated with the hydrophilicity/hydrophobicity of ILs. In hydrophobic ILs, such as [Bmim][PF<sub>6</sub>] and [Bmim][NTf<sub>2</sub>], enzymes are not soluble, and usually their powders are suspended in ILs. The suspended enzymes exhibit catalytic activity and even good stability, but only small portions are available for catalysis due to their poor dispersion. By contrast, in hydrophilic ILs, enzymes are soluble, but their activity is poor due to the unfavourable interaction between enzymes and ILs. It follows that the major problem for the utilization of ILs as media for enzyme catalysis is how to reconcile the contradiction between the maintenance of the enzyme activity and the solubility of the enzyme in ILs.

Reviewing the evolution of the medium engineering for enzyme catalysis, we get a good idea. For hydrophobic ILs (HILs), a good solution to the problem is to create a microenvironment suitable for the dispersion of an enzyme as well as the maintenance of the enzyme activity by dispersing water into HILs; i.e., the so-called microemulsification of HILs with surfactants. The microemulsification results in larger surface area than in the HIL/water two-phase system and makes the reactions of hydrophobic substrates with a hydrophilic enzyme go easily. Moreover, the formed water pool can restrict the change of the conformation of the enzyme. Studies have shown that most enzymes can maintain their catalytic activity and stability in HIL-based microemulsions. In this talk, we make a brief description of the recent progress made in my group in the enzyme catalysis in HIL-based microemulsion. To be relevant to the themes of the Conference, the talk is focused on the aggregation behaviour of different surfactants in HILs as well as the microstructural effect of the formed aggregates on solubilized enzymes.

The aggregation of surfactants of different types will be summarized. To circumvent the poor solubility of most ionic surfactants such as NaAOT in HILs, a new strategy has been developed; i.e., the substitution of the inorganic counter ion by its organic counterpart. For example NaAOT, the replacement of the counter ion Na<sup>+</sup> by [Bmim]<sup>+</sup> not only increases the surface activity of AOT<sup>-</sup> in water, but also significantly improve its solubility in [Bmim]Tf<sub>2</sub>N. Also it is found the exchange of the cations helps to formulate a W/HIL microemulsion without any additives. In addition to the construction strategy, we will present detailed studies on the regulation of the microstructure and the consequent water solubilization capacity by salts and alcohols.

The catalytic performance of enzymes hosted in HIL-based microemulsions has been characterized. It is found that the formation of the microdroplet of water in HIL facilitates the dispersion of enzymes such as laccase on a molecular level and also greatly reduces the negative effect of the ionic liquid on the enzyme. The catalytic activity of an enzyme hosted in the droplet depends upon the size of the droplets, the interfacial components and charge density. For a given enzyme, an optimal microenvironment could be created via the formulation optimization. All results indicate that HIL-based reverse micelles or microemulsions, which are homogeneous macroscopically but microscopically heterogeneous, are promising media for an enzyme catalyzed reaction