Biocatalysis

Deep Eutectic Solvents

Trial Pack

ScioniX
Ionic Liquid Technology

C-Tech Innovation
...advantage through technology

Ionic Liquids

Ionic Liquids are salts that are liquid below 100°C. They are primarily large quaternary ammonium cations (R_4N+) with large anions such as PF_6- and BF_4- . The symmetry of the ions is also important; non-symmetrical ions are more difficult to fit into a lattice and hence the lattice energy and melting point will be lower. A large amount of work has been carried out on compounds known as imidazomium salts e.g. butyl-methyl imidazolium hexafluorophosphate (BMIM PF_6). This compound is liquid at room temperature (in fact it freezes at -40°C).

Potential Benefits

Some of the potential benefits of carrying out biocatalytic processes in ionic liquids include;

- alternatives to freeze-drying and potential for enzyme storage.
- extended enzyme stability
- potential for product selectivity
- high substrate solubility

Ionic liquids are of interest because they have negligible vapour pressure, are non-flammable and have unusual solvent properties. They are, however, quite difficult to make and is therefore extremely expensive. The toxicity of imidazolium salts has also not yet been ascertained.

Use of Deep Eutectic Solvents for Biocatalysis

DESs and Ionic liquids can be used like any other non-aqueous solvent for biocatalysis. Generally the enzyme is added in a concentrated aqueous buffer such that the aqueous component is less than 1 wt. % of the mixture. The substrate is then stirred into the mixture as normal. Extraction of products can be made using a variety of solvents. The following are all immiscible with all of the DESs in the pack; acetonitrile, ether, ethyl acetate, acetone, dichloromethane and hexane.

DESs are super-cooled liquids and their freezing point can be significantly lower than their melting point. This allows them to be stored as a solid. We found that α-Chymotrypsin could be dissolved in anhydrous Reline and once frozen the mixture could be returned to ambient temperature and stored. When the mixture was melted after a week it was found to retain the majority of its activity.

The DESs in this pack have different solvent properties and may not be suitable for all types of biocatalysis, but the range of liquids will provide some scope of what is possible.

Biocatalysis in Ionic Liquids

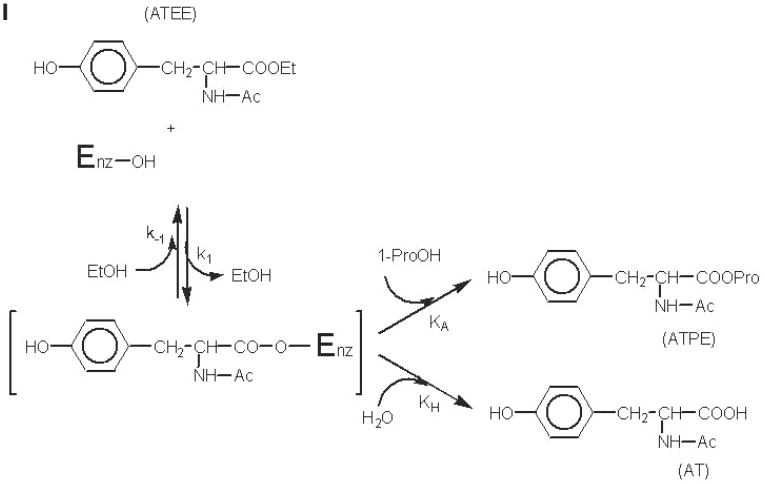
Enzymes have been shown to be stable in ionic liquids and the work to date has recently been reviewed.** While a variety of biotransformations have been shown to be possible using ionic liquids, this is a very new field and no concerted studies have as yet been carried out.

One example of biocatalysis in a DES is the proteolytic enzyme α-Chymotrypsin which can act on *N*-acetylated esters of its substrates, such as the *N*-acetyl-L-tyrosine ethyl ester shown in Figure 1.

Hydrolysis reaction predominates in aqueous environment. In other solvent systems, the enzyme can be used to transesterify the substrate with propanol, to form *N*-acetyl-L-tyrosine propyl ester. This reaction has been carried out in Reline and it was found that the enzyme is stable for several days and yields primarily the transesterified product

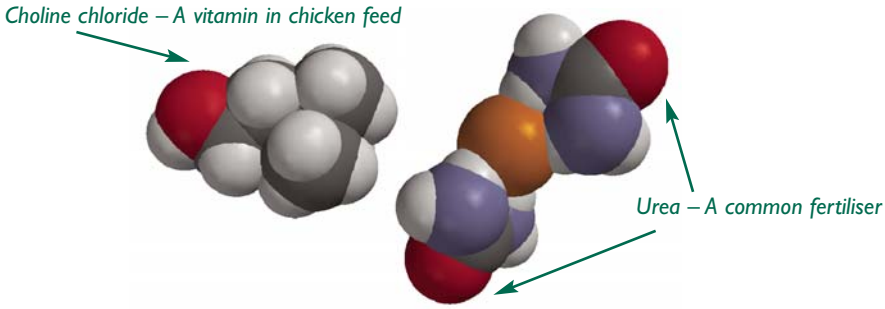
Although it may seem counter-intuitive that enzymes are stable in high chloride containing media it must be remembered that the chloride is strongly hydrogen bonded with urea and therefore does not interact with the enzyme.

Figure 1



Ref: ** Wasserscheid, P.; Welton, T. *Ionic Liquids in Synthesis* Wiley-VCH Verlag, Weinheim, Germany 2003.

Another alternative approach to making ionic liquid analogues is to take a simple organic halide salt and complex the anion with something that will form a hydrogen bond. The complexing agent will decrease the interaction between the anion and the cation and decrease the freezing point of the mixture. To differentiate these liquids from the more commonly studied imidazolium salts the term **Deep Eutectic Solvents** (DES) has been coined. This approach is not only cheaper and easier to make, but many of the formulations are non-toxic and even biodegradable. A wide variety of salts can be used with an even wider range of hydrogen bond donors. This trial pack contains four different DESs with significantly different solvent properties.*



An example of this is choline (2-hydroxyethyl-trimethylammonium) chloride (this is vitamin B4 and is produced on the Mtonne p.a. scale) mixed with urea ($NH_2C{=}ONH_2$)

DES are simple to make – just mix the two components

Ref: *. *Abbott, A. P. et al., J. Am. Chem. Soc., 2004, 126, 9142*

Trial Pack contains 4 Deep Eutectic Solvents

	Constituents
Reline	Choline Chloride and urea
Maline	Choline chloride and malonic acid
Ethaline	Choline chloride and ethylene glycol
Glycaline	Choline chloride and glycerol

To order a trial pack of Deep Eutectic Solvents or to find out more about Ionic Liquids and Deep Eutectic Solvents. See below for contact details:

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