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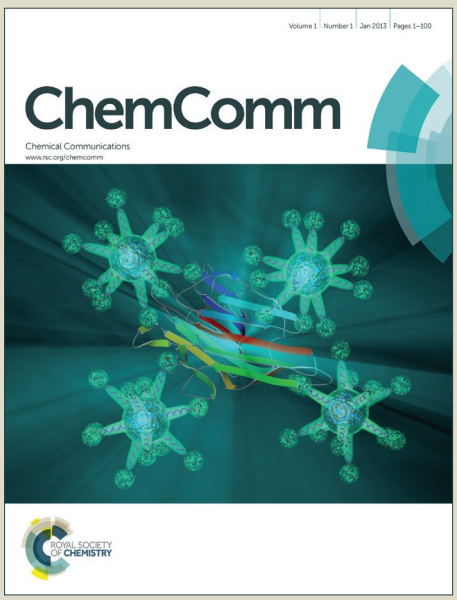

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FEATURE ARTICLE

Active biopolymers in green non-conventional media: A sustainable tool for developing clean chemical processes

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Pedro Lozano,*[a] Juana M. Bernal,[a] Susana Nieto,[a] Celia Gomez,[a] Eduardo Garcia-Verdugo[b] and Santiago V. Luis[b]

The greenness of chemical processes turns around two main axes: the selectivity of catalytic transformations, and the separation of pure products. The transfer of the exquisite catalytic efficiency shown by enzymes in nature to chemical processes is an important challenge. By using appropriate reaction systems, the combination of biopolymers with supercritical carbon dioxide ($scCO_2$) and ionic liquids (ILs) resulted in synergetic and outstanding platforms for developing (multi)catalytic green chemical processes, even under flow conditions. The stabilization of biocatalysts, together with the design of straightforward approaches for separation of pure products including the full recovery and reuse of enzymes/ILs systems, are essential elements for developing clean chemical processes. By understanding structure-function relationships of biopolymers in ILs, as well as for ILs themselves (*e.g.* sponge-like ionic liquids, SLILs; supported ionic liquids-like phases, SILLPs, etc.), several integral green chemical processes of (bio)catalytic transformation and pure product separation are pointed out (*e.g.* the biocatalytic production of biodiesel in SLILs, etc.). Other developments based in DNA/ILs systems, as pathfinder works for further technological applications in the near future, are also considered.

1. Biopolymers for sustainable chemical processing

The sustainability of chemical processes begins with catalysis, because the selectivity in chemical transformations is directly related with several of the principles of green chemistry, *i.e.* prevention, atomic economy, less hazardous synthesis, reduction of derivatives, etc.[1] The inherent formation of wastes/contaminants and undesired by-products in classical synthetic processes, which are based on the use of stoichiometric amounts of reagents, can be minimized by using catalytic steps.[2] The development of efficient catalytic processes leads to significant savings in production costs for industry, as well as in environmental impacts. Other greener aspects to be taken into account for developing clean chemical processes also include the use of bio-renewable raw materials[3] in benign reaction media,[4] even under flow conditions,[5] as well as alternative reaction activation methodologies, *e.g.* microwave and ultrasonic irradiations, etc., for improving catalytic efficiency over conventional energy sources.[6] All these key concepts may be integrated for developing selective processes of transformation and separation, able to directly provide pure products, including the reuse of all the elements of the reaction system, *e.g.* catalysts, solvents, etc.

Nature has always been a source of inspiration for chemists. Catalytic transformations in living systems are carried out by biopolymers (*i.e.* enzymes and RNAs), being the most sustainable catalysts for chemical processes, because they are biocompatible, biodegradable, and are derived from renewable resources. The pioneering work of Edward Buchner, Nobel Prize in Chemistry 1907, demonstrated the ability of an extract of died yeasts for carrying out the alcoholic fermentation, a ten-step biocatalytic path for transforming glucose to ethanol.[7] This discovery was determinant not only to bury the theory of vitalism, but also to highlight the suitability of *zymases* (enzymes) for developing multi-catalytic processes of industrial interest. Enzymes, usually named biocatalysts, clearly constitute a powerful green catalytic toolbox for chemical processes. A great variety of enzyme-catalyzed reactions have been successfully demonstrated at laboratory scale, offering clear advantages for the synthesis of enantiopure fine chemicals against any other kind of catalysts.[8] Developments in genomics, directed evolution and our exploitation of the natural biodiversity have led to improvements in the activity, stability and specificity of enzymes, accompanied by a huge increase in the number and variety of their industrial applications.[9] Furthermore, the technological applications of enzymes are greatly enhanced in non-aqueous environments, rather than in their natural aqueous reaction media, because of the catalytic promiscuity that results in the expansion of the repertoire of biotransformations.[10] Among the most used enzymes, lipases have gained a clear predominance, exhibiting a wide specificity to recognize very different substrates, and catalyzing different reactions used in pharmaceuticals and drugs synthesis,[11] biofuels,[12] food and cosmetic additives,[13] etc.

[a] Departamento de Bioquímica y Biología Molecular "B" e Inmunología. Facultad de Química. Campus de Excelencia Internacional Mare Nostrum, Universidad de Murcia. E-30.100. Murcia. Spain. E-mail: plozanor@um.es.
[b] Departamento de Química Inorgánica y Orgánica, Universidad Jaume I, Campus del Riu Sec, Avenida Sos Baynant s/n, E-12071, Castellón, Spain.

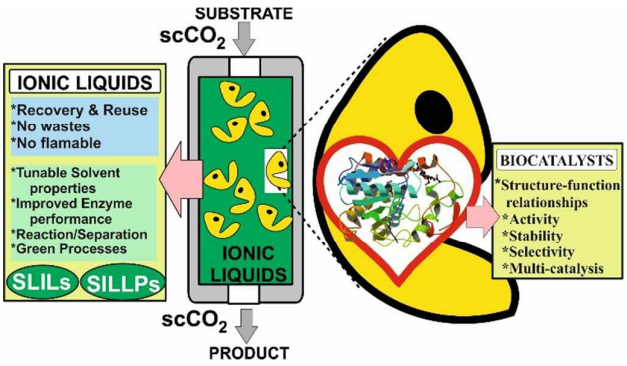

Fig. 1. Key axes for developing green synthetic processes based on biocatalysis and ionic liquids (*e.g.* SLILs: sponge-like ionic liquids; SILLPS: supported ionic liquid-like phases).

The appropriate selection of non-aqueous reaction media is key for the success of biocatalytic processes, although switching from water to non-aqueous solvents is not always a simple answer because of enzyme deactivation.[10] In this respect, ionic liquids (ILs)[14] and supercritical carbon dioxide ($scCO_2$)[15] are the non-aqueous green solvents that have received most interest to design sustainable approaches of (bio)catalytic transformations and product separation, even under flow operation.[5]

The aim of this perspective article is to understand the structure-function relationships of biocatalysts in ILs, or ILs/$scCO_2$ biphasic systems for developing new platforms of chemical synthesis. The structural organisation, solvent properties and phase behaviour of ILs, as well as the interactions established with enzymes for the maintenance of their catalytic functions are key concepts to be considered for developing clean chemical processes (see Fig. 1). Different examples concerning the excellent suitability of some tailor-made ILs, *e.g.* sponge-like ionic liquids (SLILs), supported ionic liquid-like phase (SILLPs), etc., for carrying out integrated (bio)catalytic transformation and downstream processes are highlighted. The synergic combination of both the biological systems and green solvents in appropriate reactors (e.g. membrane reactors, continuous ILs/$scCO_2$ biphasic reactors, etc.), might be considered as an efficient toolbox for pushing up clean chemical processes of industrial interest, being able to provide directly pure products, and to allow an easy recovery of the IL/biocatalysts systems for further reuse. Developments based in nucleic acids/ILs systems, as pathfinder works for further technological applications in the near future, are also discussed.

2. Understanding enzyme function in ionic liquids.

All enzymes are proteins, polymeric macromolecules based on a unique sequence of amino acid units, which show a high level of 3-D structural organization, also named conformation. The maintenance of the native conformation is dynamically carried out by a high number of weak intramolecular interactions (*e.g.* hydrogen bonds, van der Walls, etc.), as well as interactions with other molecules, mainly water as natural solvent for life.[16] Indeed, enzymes are designed by cells to function in aqueous media within a narrow range of environmental conditions (*e.g.* temperature, pH, etc.), which in fact represent the limits of life. Outside these conditions, enzymes usually become inactive because of a loss in native conformation through unfolding. Furthermore, the tremendous potential of enzymes as practical catalysts for chemistry, determined by their high level of activity and selectivity (stereo-, chemo- and regioselectivity), is also fully dependent on the maintenance of its native conformation. Enzymes typically fold in such a way that non-polar residues are buried in a hydrophobic core, while polar residues tend to move to the surface, where they are hydrated. In all non-conventional media, water is a key component for maintaining the active conformation of enzymes. Dry proteins are completely inactive and a few clusters of water molecules are required for the catalytic function. Thus, the internal structures at the active site of proteins are constantly shaped by strong interactions with the hydration shell and bulk water motions.[17] In this context, hydrophilic solvents strip the essential water molecules interacting with enzymes, resulting in fast biocatalyst deactivation.[18] On the contrary, water-immiscible solvents typically afford higher enzymatic activity than hydrophilic ones, being assumed that a hydrated enzyme placed in a dry hydrophobic system is trapped in the native state, being able to maintain its catalytic activity.[10]

Ionic liquids are exceptional non-aqueous reaction media for carrying out both chemocatalytic[19] and biocatalytic processes.[14,20] They are liquids at temperatures lower than 100ºC, which are composed entirely by ions, and their use has led to a green chemical revolution because of their unique array of physical-chemical properties (i.e. low vapour pressure, non-flammable nature, high ionic conductivity, good dissolution power towards many substrates, high thermal and chemical stabilities etc.).[21] Typical ILs used in biocatalytic processes are based on organic cations, *e.g.* dialkylimidazolium, tetraalkylammonium, etc., paired with anions that have a strongly delocalized charge (*e.g.* [PF_6], bistriflimide, etc.). In contrast to conventional organic solvents, the green label of ILs is headed by its non-volatile character, which permits their full recovery for further reuse. By the appropriate choice of cation and anion, the polarity and hydrophilicity/hydrophobicity of ILs can be tuned, modifying its miscibility with molecular solvents (*i.e.* water, organic solvents, etc.), which has been applied for developing useful approaches of products recovery from the reaction mixture. Ionic liquids, even those non-miscible with water, are polar and hygroscopic solvents that can absorb a few percent of water.[22] As a representative example, the 1-butyl-3-methylimidazolium bistriflimide ([Bmim][NTf_2]) is a water-immiscible IL able to dissolve water molecules up to 1.4% (w/v),[23] which is an interesting property to understand its excellent suitability for biocatalysis. The presence of essential water molecules in ILs is key for the maintenance of native protein conformations in biocatalytic applications.

Although research on enzyme-catalyzed reactions in ILs only began in 2000,[24] the use of such neoteric solvents in biotransformations has increased exponentially to more than 3,000 published papers until today.[25] Researchers have first focused on the suitability of ILs as reaction media for biocatalysis, then on the understanding the

exceptional behaviour of biocatalysts in some kinds of ILs, and finally on the development of integrated green processes for biotransformation and product separation.[4,5,14,20,26]

For the case of water-miscible ILs, the biocatalytic activity is highly dependent on the nature and concentration of ions, because of the ability of these ILs to deactivate enzymes by water stripping. As an example, the alcalase-catalysed enantioselective resolution of N-acetyl amino acids was improved greatly by using 10% v/v of N-ethylpyridinium trifluoro acetate ([EPy][TFA]), but drastically fall at higher IL concentration.[27a] In another example, the best activities for the laccase-catalysed oxidation of catechol in [Bmim][Br] and [Bmim][$N(CN)_2$] were observed at concentrations between 10-20% and 50-60% (v/v) in water, respectively, while the activity was decreased at higher and lower concentrations.[27b] A similar behaviour of activity decay was observed for other enzymes (*e.g.* chloroperoxidase, formate dehydrogenase, β-galactosidase, etc.), being also attributed to the water stripping produced by water-miscible ILs.[14]

On the other side, all the assayed water immiscible ILs (*e.g.* [Bmim][NTf_2], [Bmim][PF_6], [Btma][NTf_2], etc.) have been shown as suitable reaction media for biotransformations, appearing as viable alternatives to molecular organic solvents for organic synthesis.[4,5,12,14,20,26] At low water content, these ILs provide an appropriate microenvironment to enzymes because of the preservation of the essential hydration shell, resulting in an enhancement in activity and stability.[28,29] Thus, for example, studies on the secondary structures of different proteins (*i.e.* monellin,[29a] α-chymotrypsin[29b] and CALB[29c]) in several water-immiscible ILs (*e.g.* [Bmim][NTf_2], [Btma][NTf_2], [Bmpy][NTf_2], etc.), carried out by both circular dichroism and fluorescence spectroscopies, allowed to correlate the stabilization phenomena of ILs with the preservation of the native structure of the enzymes, which displayed flexible and active conformations. Water-immiscible ILs form a strong ionic matrix which retains enzyme molecules in an adequate microenvironment, resulting in a supramolecular net able to keep the protein conformation active because of the preservation of its essential water shell. In this context, ILs were also used as coating agents for enzymes stabilization in non-aqueous environment resulting in biocatalysts derivatives with improved performance in activity, stability and enantioselectivity.[30] As an example, the IL [1-(3'-phenylpropyl)-3-methylimidazolium)][PF_6], which is solid at room temperature and becomes liquid above 53 °C, was used to coat free *P. cepacia* lipase in the liquid phase. Afterwards, the mixture was cooled and then, by cutting the solid mixture into small pieces, a useful immobilized enzyme derivative was obtained with markedly enhanced enantioselectivity and without losing any significant activity towards reuse.[31] In another example, it was observed how the activity and stability of cellulase against 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]), a water-miscible IL that produces fast enzyme deactivation,[32] can be greatly enhanced by coating the immobilized enzyme particles with hydrophobic/water-immiscible ILs (*e.g.* [Bmim][NTf_2], etc.).[33] By this approach, cellulase was able to catalyse saccharification of cellulose solutions in ([Bmim][Cl] at 50ºC (see Fig. 2). In the same

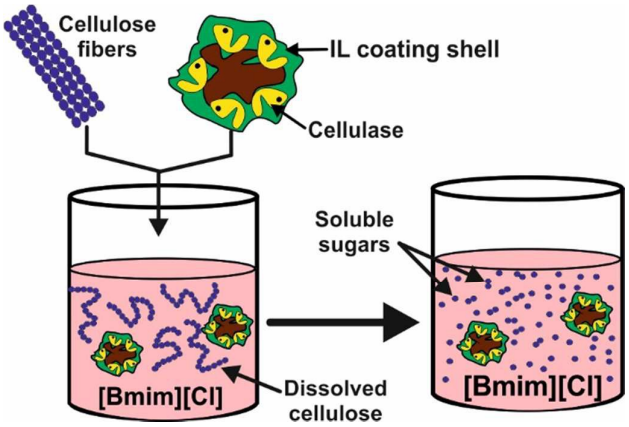

Fig. 2. Saccharification of cellulose dissolved in [Bmim][Cl] catalyzed by immobilized cellulase coated with a hydrophobic IL, *e.g.* [Bmim][NTf_2]. *Reproduced from Ref. 33 with permission from the Royal Society of Chemistry*.

context, it was also reported how ILs composed of hydrophobic but hydrated ions (*i.e.* more than seven water molecules per ion pair) were successfully applied to the separation of active water-soluble proteins by dynamic phase change of these ILs.[34]

All these phenomena have been attributed to the unique structural characteristic of ionic liquids.[4a,14,20] Dupont has described the structural organization of imidazolium ILs in solid and liquid phases, [35] where these ILs form an extended network of cations and anions connected together by hydrogen bonds (see Fig 3A). The monomeric unit is always constituted by one imidazolium cation surrounded by at least three anions and, in turn, each anion is surrounded by at least three imidazolium cations, where the strongest hydrogen bond always involves the most acidic H, placed at C-2 position of the imidazolium ring. The supramolecular net is also maintained by the typical π/π stacking interactions between heterocyclic rings. Thus, the incorporation of other molecules into the IL network induces changes in the structural organisation, and in some cases (*e.g.* water) may cause the formation of polar and nonpolar regions. Wet ILs are shown as nano-structured materials, which allow neutral molecules to reside in less polar regions, and ionic or polar species to undergo faster diffusion in the more polar or water rich regions.[35a] Accordingly, enzymes in water immiscible ILs should also be considered as included into hydrophilic gaps of the network, where the observed stabilization of enzymes may be attributed to the maintenance of this strong net around the protein (see Figure 3B). Furthermore, considering a classical enzyme process that occurs in water, by increasing temperature,[36] the protein unfolding could also be attributed to the disruption of the structural organisation of the medium, as a consequence of the increase in the kinetic energy of water molecules when heated.[37] The extremely ordered supramolecular structure of ILs in liquid phase may be able to act as a "mould",[38] maintaining an active three-dimensional structure of the enzyme in aqueous nano-environments, and avoiding the classical thermal unfolding.[4,28,39]

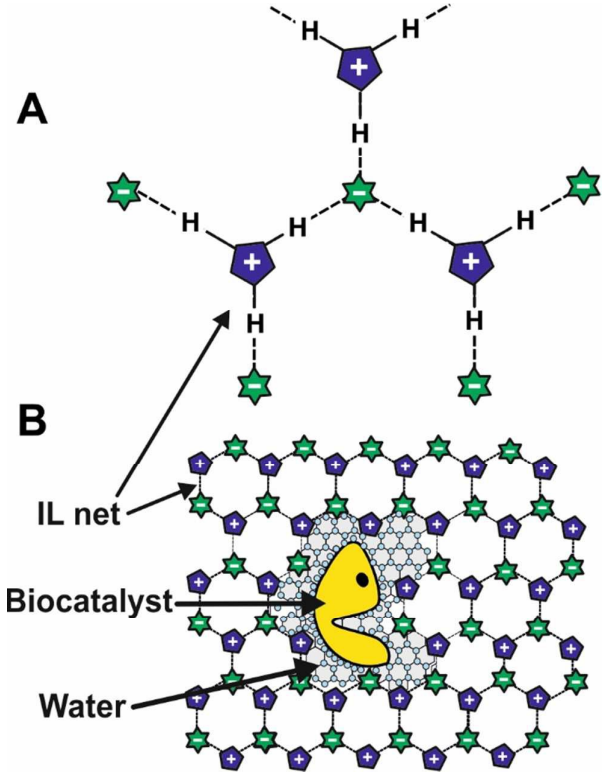

Fig. 3. **A**. Model of the supramolecular structure of imidazolium ILs net based on hydrogen bonding interactions.[35] **B.** Active enzyme with native folded conformation into a wet gap of water-immiscible IL network.[20,28]

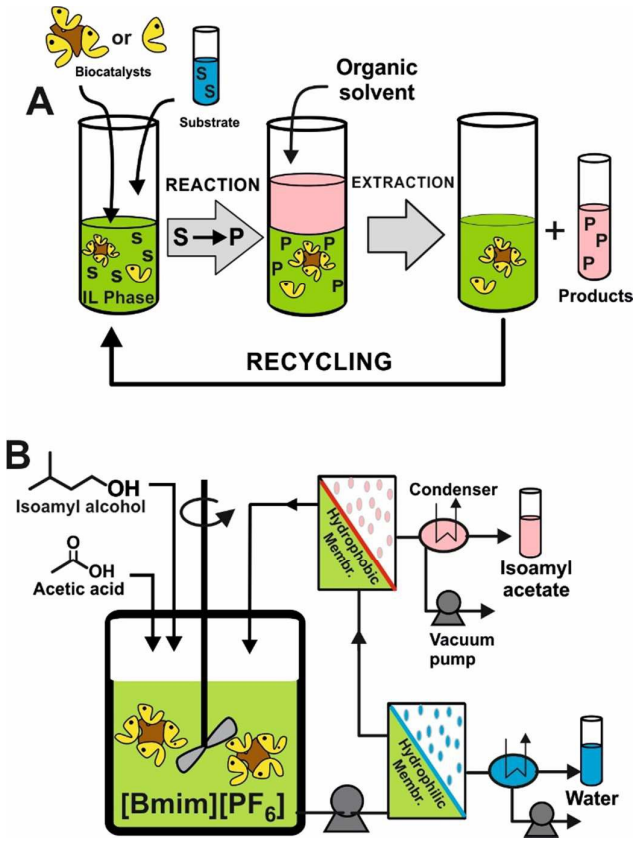

Fig. 4. **A**. Enzyme-catalyzed reactions in IL medium including product recovery by liquid-liquid extraction with an organic solvent and recycling of the enzyme/IL system. **B.** Immobilized lipase-catalyzed isoamyl acetate synthesis in [Bmim][PF_6] using a stirred tank reactor coupled to pervaporation units for products separation.[45a]

Furthermore, an additional new concept should be discussed. Classically, immobilized enzymes obtained by chemical or physical attachment onto solid supports have been considered advantageous over free enzyme molecules, because they facilitate the recovery and reuse of the biocatalyst, allowing continuous processes to be designed.[40] However, in the case of water-immiscible ILs, free enzyme molecules suspended in these media behave as anchored or immobilized biocatalysts, because they cannot be separated by liquid-liquid extraction (*i.e.* with buffer or aqueous solutions). Thus, these water-immiscible ILs should be regarded as being a liquid immobilization supports, rather than just a reaction medium.[20,28a] To eliminate protein molecules from ILs, it is necessary to filter the enzyme-IL solution through ultrafiltration membranes with a cut-off lower than the molecular weight of the enzyme.[41]

3. Clean biocatalytic processes in Ionic Liquids.

To build a green chemical industry it is necessary to develop integrated processes of selective transformation and separation capable of directly providing pure products, including the reuse of all the elements of the reaction system, *e.g*. catalysts, solvents, etc. The excellent suitability of ILs as non-aqueous reaction media for enzyme catalysis has been widely described, being observed significant improvements in activity and selectivity with respect to those observed in organic solvents. The most popular experimental approach consists on the direct addition of biocatalysts to the IL medium containing substrates, and then products are recovered by liquid-liquid extraction with volatile organic solvents, while the IL/biocatalysts system is reused (see Fig. 4A).[14,24,28,41,42] Using this approach, the overall greenness of the process fails greatly, as they often require the consumption of large amounts of environmentally non-benign solvents, being necessary to find alternative strategies for product recovery based on sustainable tools (*e.g.* membrane technology, etc.).

Solute extraction from ILs by membrane technology is one of the most promising sustainable approaches for developing clean biocatalytic processes. The pioneering work of Afonso *et al* [43] demonstrate the excellent suitability of pervaporation techniques for the quantitative and selective recovery of volatile compounds (e.g. ethyl hexanoate, etc.) from ILs (*e.g.* [Bmim][PF_6], etc). The separation principle of pervaporation is based on the preferential partitioning of a solute from a liquid feed phase into a dense, non-porous membrane through which it diffuses according to its chemical potential gradient pushed by vacuum.[44] This approach has been applied for the enzymatic synthesis of flavour esters (*e.g.* ethyl acetate, isoamyl acetate) by esterification in ILs as reaction media.[45] This is illustrated

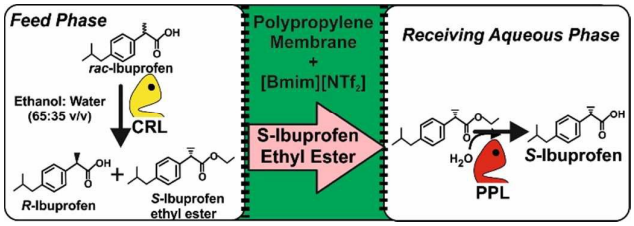

Fig. 5. Schematic diagram of enantioselective transport of (S)-ibuprofen through a lipase-facilitated supported liquid membrane (SLM) based on ionic liquids (*C. rugosa* lipase, CRL; porcine pancreas lipase, PPL).[47a]

in Fig. 4B for the immobilized CALB-catalyzed esterification of acetic acid and isoamyl alcohol in a stirred-tank reactor containing [Bmim][PF_6] as reaction medium. By coupling two pervaporation membrane units in the reactor system, using hydrophobic and hydrophilic membranes, respectively, it was possible to remove both the isoamyl acetate (100% conversion yield) and water products in a continuous operation for 72 hours, and without any loss in the enzyme activity.[45] In the same context, ILs were also used to prepare supported liquid membranes (SLM) for the selective transport of organic molecules with representative organic functional groups (*e.g.* hexylamine, methylmorpholine, etc.). The appropriate combination of selected ILs and supporting material was crucial for achieving good selectivity in separations.[46] The combination of this approach with biocatalysis was shown to be a useful tool for the clean separation of organic acids (*e.g.* 4-phenoxybutyric acid, 3-phenoxypropionic acid, etc.), and racemic mixtures of organic compounds (*e.g. rac*-ibuprofen).[47] By using water immiscible ILs (*e.g.* [Bmim][NTf_2]) in a SLM, the system permits the selective separation of a target molecule by exploiting the solubility differences between SLM and feed/receiving liquid phases, as it was smartly demonstrated for the kinetic resolution of *rac*-ibuprofen (see Fig. 5).[47a] For this example, the membrane reactor was operated by coupling two lipase reactions (esterification and hydrolysis, respectively) placed at each one of the side of the membrane. The IL-based SLM permits the selective transport of the synthesized *S*-ibuprofen ester from the feed phase to the receiving aqueous phase, where the ester product was then hydrolysed by another lipase. By this approach, a clean resolution of the racemic mixture was carried out by using the enzyme-facilitative SLM system.

A further step towards the development of clean approaches for pure product recovery from ILs phases has been reported recently, because of the discovery of its sponge-like behaviour (i.e. sponge-like ionic liquids, SLILs).[20,48] This terms refers to water-immiscible ILs with long alkyl side-chains in the cations (*e.g*. octadecyltrimethylammonium bistriflimide [C_{18}tma][NTf_2],[49] 1-ocadecyl-3-methylimidazolium bistriflimide ([C_{18}mim][NTf_2],[50] etc., that behave as temperature switchable ionic liquid/solid phases. It was described how these ILs are able to dissolve liquid hydrophobic compounds (e.g. triolein, geraniol, nerol, citronellol, etc.), forming monophasic liquid systems upon heating above their melting points (see Fig. 6A). Then, these fully clear liquid solutions, containing both the compound and the IL, became monophasic solid systems after

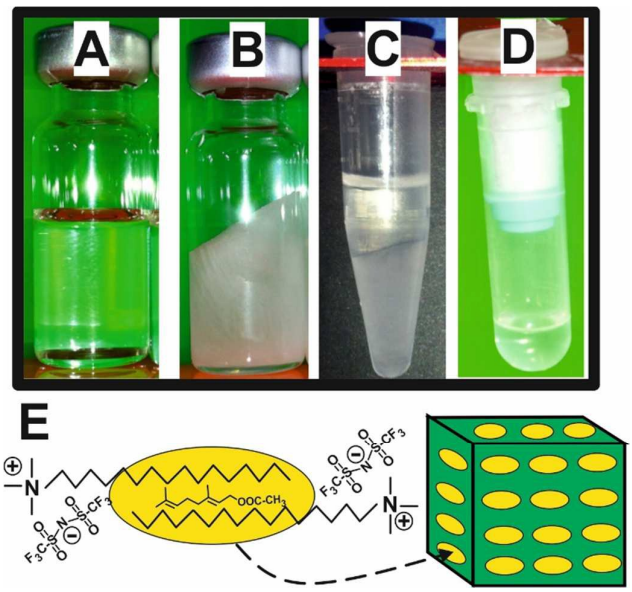

Fig. 6. Phase behaviour of 50/50 (w/w) neryl acetate/[C_{16}tma][NTf_2] mixture at 50ºC (**A**), 25 ºC (**B**), and after four consecutive centrifugation steps at 14,000 rpm (15 mim) at room temperature, 21, 10 and 4ºC (**C**), respectively. (**D**) Phase behaviour of 30/70 (w/w) anisyl acetate acetate/[C_{16}tma][NTf_2] mixture after centrifugation at 16,000 rpm (15 mim) through a nylon filter (0.2μm pore size). *Reproduced from Ref. 20 with permission from the Royal Society of Chemistry* (**E**) Hypothesis of the structural organization of the solid [C_{16}tma][NTf_2] net (green) with hydrophobic holes (yellow) containing liquid geranyl acetate *Reproduced from Ref. 50a with permission from the Royal Society of Chemistry*.

cooling down to room temperature (see Fig. 6B). Furthermore, these solid phases can be separated into two phases by simple centrifugation at a temperature lower than room (see Fig. 6C). The upper liquid phase was an IL-free flavour ester, as determined by ^{19}F NMR studies,[49b] and the bottom solid was the solid IL. The separation can be improved even further by introducing the solid mixture, formed by the hydrophobic compound and the IL into a nylon centrifugal filter (0.2 μm pore size). Then, the application of an external force at low temperature (*e.g.* 10 min centrifugation, 16,000 rpm, 0ºC) led to the retention of the solid IL phase above the nylon membrane, while the liquid flavour was filtered through the membrane to the bottom of the tube (see Fig. 6D), as though wringing out a sponge.[50b] This unique feature was explained as a function of a sponge-like behaviour of these temperature switchable ionic liquid/solid phases, where flavour esters were considered as being included rather than dissolved in the liquid/solid IL phases. The decrease in free volume of the ionic net produced by cooling allowed compaction of the IL solid phase by centrifugation, and the consequent release of flavour molecules outside the net. Thus, the IL net could be considered as a nano-sponge with holes of variable volume, which are suitable for housing or releasing hydrophobic molecules as a function of their liquid or solid phase, respectively (see Fig. 6E).

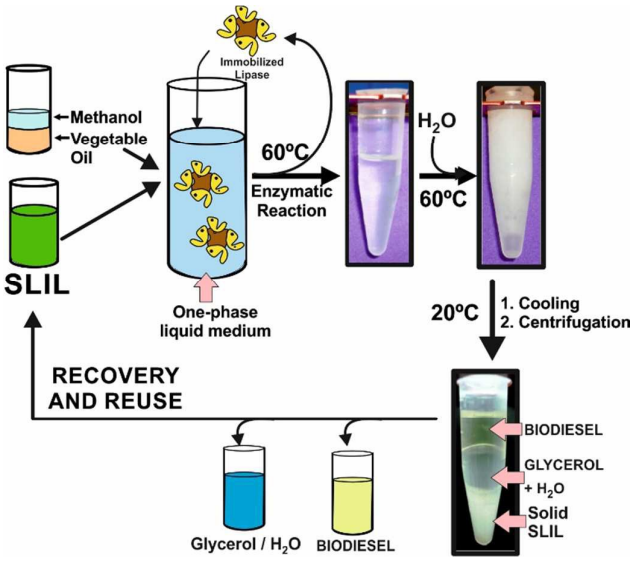

Fig. 7. Cyclic protocol for the clean biocatalytic production of pure biodiesel in sponge-like IL (SLIL), including the full recovery and reuse of the enzyme/SLIL system.[48]

As an illustration of this strategy, the enzymatic production of biodiesel in sponge-like ILs is a good example of clean biocatalytic process.[48] Biodiesel is usually synthesized by transesterification of triacylglycerides with methanol, yielding fatty acid methyl esters, and glycerol as by-product by using either chemical (e.g. KOH) or biological (*e.g.* lipases) catalysts. However, the non-miscibility between both vegetable oil and methanol substrates is a key feature for reducing the efficiency of the catalysts in biodiesel synthesis and, for the case of biocatalysis; this also causes full and fast enzyme deactivation as a result of the direct interaction between the catalytic protein and the methanol phase. In this context, it was reported how ILs based on cations with long alkyl side-chains (*e.g.* $[C_{18}mim][NTf_2]$, $[C_{18}tma][NTf_2]$, etc.) were able to dissolve both triolein and methanol at any concentration, providing one-phase reaction media that showed excellent suitability for the biocatalytic synthesis of biodiesel, *i.e.* up to 96% yield in 6 h at 60ºC. Furthermore, the excellent suitability of the enzyme/IL system for biodiesel synthesis was also demonstrated by the total preservation of the catalytic activity for subsequent reuse.[48,51] Furthermore, an straightforward and clean cyclic protocol was proposed for the extraction of both the synthesized biodiesel and the by-product glycerol from the reaction mixture as a result of the sponge-like behaviour of these hydrophobic ILs (see Fig. 7). Thus, an initial washing-by-water step on the reaction media as a liquid phase before cooling to room temperature was carried out. The resulting semisolid heterogeneous mixture was then separated by following an iterative cooling/centrifugation protocol, which resulted in three phases: an upper IL-free biodiesel phase, a middle IL-free liquid aqueous phase containing the glycerol, and a bottom solid containing the SLIL. The presence of water, a green molecular solvent (non-miscible with the biodiesel and the SLIL), improved the separation of all phases, providing an easy and sustainable way to separate both glycerol and non-reacted methanol from the biodiesel. The suitability of the proposed clean approach for biodiesel synthesis was demonstrated by the full recovery and reuse of the SLIL/biocatalyst system. This opens up a new road in green chemistry for separating products from reaction media based on ILs.

4. Clean biocatalytic processes in supercritical fluids.

Supercritical fluids (SCFs) are another environmental friendly alternative to organic solvents as media for developing clean biocatalytic processes. A SCF is defined as a state of matter at a pressure and temperature higher than its critical point, but below the pressure required to condense it into a solid. These fluids are characterized by gas-like viscosities, and solvating properties in a wide range covering those of several organic solvents. Tuning physical properties of these solvents simply by adjusting the pressure and temperature is unique to supercritical systems, which show exceptional abilities for extraction, reaction, fractionation and analysis processes. [52]

As regards biocatalysis, it is well known that proteins are insoluble in all SCFs, which permits them to be easily recovered and reused, while the mass-transport rates of reactants to the active site of enzymes are enhanced in these solvents. Furthermore, products can be easily freed from solvent traces, because SCFs are removed by simple depressurisation.[53] If the appropriated pressure and temperature conditions are selected, enzymes are catalytically active in SCFs.[54] This fact involves that SCFs with low critical temperature are required, as proteins tend to denature at high temperature. Although CO_2, ethane, propane, butane, SF_6, CHF_3, are typical fluids suitable for biocatalysis under supercritical conditions, the supercritical carbon dioxide ($scCO_2$; P_c = 73.8 bar; T_c = 31.0ºC) remains the most popular SCFs for biocatalysis by far because it is chemically inert, non-toxic, non-flammable, cheap and readily available.[55] Although $scCO_2$ is considered as a hydrophobic solvent (*e.g.* 0.31% w/w water content at 344.8 bar and 50ºC), the capability of $scCO_2$ to strip the essential water molecules from the enzyme microenvironment was suggested as being responsible for the observed low activity and fast deactivation.[53,56] Furthermore, its chemically inert character could be in doubt regarding its interaction with proteins, because CO_2 forms carbamates with ε-amine groups of lysine residues placed on the enzyme surface, and decreases the pH of the aqueous layer around the enzyme. Both phenomena have been directly related with the usual enzyme deactivation observed in $scCO_2$.[54,57] Enzyme stabilization approaches based on the enzyme immobilisation onto a support have become an essential tool for developing biocatalytic processes in SCFs. As an example, the covalent attachment of enzymes onto inorganic materials (*e.g.* alumina ceramic membranes, etc.), previously coated with hydrophilic polymers (*e.g.* polyethyleneimine, etc.), was then applied in a cross-flow filtration unit for producing flavour esters (*e.g.* butyl butyrate, etc.) under $scCO_2$ conditions, resulting in an enhancement in the activity and the operational stability of the biocatalyst.[58]

Although biocatalytic processes in SCFs can be performed in high pressure batch vessels, continuous flow processes are more

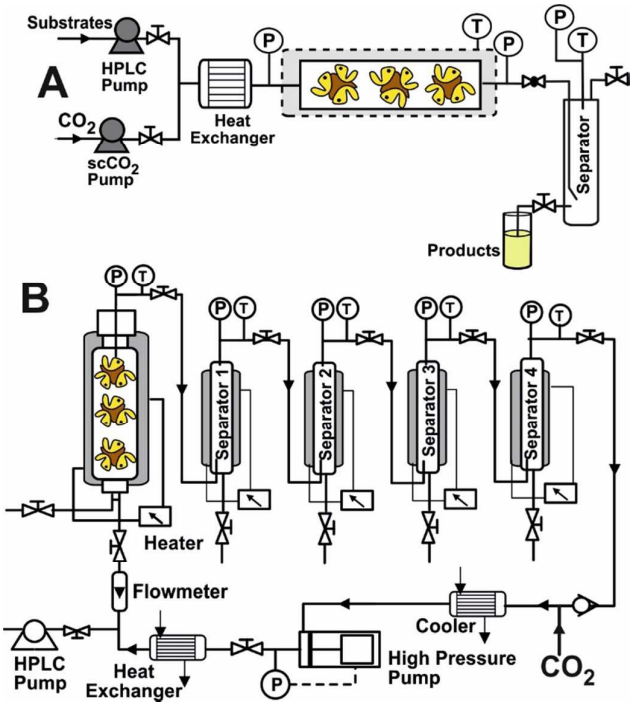

Fig. 8. **A**. Continuous flow packed-bed reactor for the biocatalytic synthesis of octyl oleate,[61] R-1-phenhylethyl acetate[62a] geranyl acetate[62b] in $scCO_2$. **B.** Continuous process for producing of ethyl oleate by combining the continuous biocatalytic reaction and supercritical fractionation with $scCO_2$ recycling.[63]

attractive because of a series of practical and technical advantages.[5,52-55,59,60] Biocatalytic flow processes allow continuously reactor feed with substrates, facilitate mass transfer, an easy control of the experimental conditions (pressure, temperature, flow, etc.) and the product isolation and purification. As an example, the production of long-chain fatty acid esters (*e.g*. alkyl oleate) is of great interest for cosmetic, pharmaceutical and lubricant industry. Thus, the esterification between oleic acid and 1-octanol catalysed by *R. miehei* lipase (Lipozyme[RM]) immobilized on a macroporous anion exchange resin has been studied in batch and continuous packed-bed reactor in $scCO_2$, being able to reach up to 93% conversion under the optimised conditions, and the productivity was maintained for 50 days (see Fig. 8A).[61] A similar reactor design was used for commercial immobilized *C. antarctica* lipase B (Novozym 435)-catalyzed kinetic resolution of *rac*-1-phenylethanol (*i.e*. up to 99% *ee* of product),[62a] as well as for the esterification reaction of geraniol with acetic acid in $scCO_2$ (up to 85% product yield)[62b] under continuous operation. The design of such enzymatic reactor under supercritical conditions is a key feature, because mass-transfer limitations, environmental conditions (pressure and temperature) and product recovery can easily be controlled to improve the results. In another example, a recycling packed bed enzyme-reactor at pilot plant scale is designed for the Lipozyme[RM]-catalyzed ethyl oleate synthesis by esterification from oleic acid and ethanol in $scCO_2$ (see Fig. 8B).[63] The proposed system was coupled with a series of four high-pressure separator vessels, where a pressure cascade was produced by back-pressure valves, allowing continuous

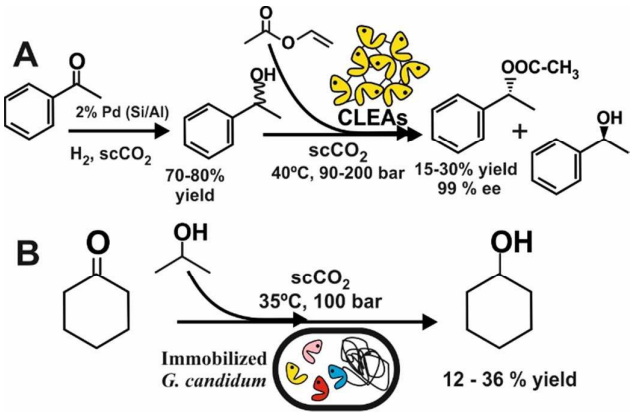

Fig. 9. **A**. Pd-catalyzed hydrogenation of acetophenone coupled with Cross-Linked Enzyme Aggregates (CLEAs)-catalyzed kinetic resolution (KR) of the resulting *rac*-1-phenylethanol in $scCO_2$.[65] **B**. Biocatalytic reduction of cyclohexanone in $scCO_2$.[66]

recovery of the liquid product at the bottom of each separator, and recycling of untransformed substrates and CO_2.

Alternatively, packed bed reactors containing supported reagents, scavengers and (bio)catalysts can be easily set-up in flow sequential assembling under supercritical conditions, opening the way to produce multistage chemical reactions.[64] This enables the design of cascade multiple-stage chemocatalytic and biocatalytic processes, and easily increases of the complexity and value of the synthesized products. In this regard, Poliakoff and co-workers reported a tandem palladium (Pd)-catalysed hydrogenation of acetophenone followed by a lipase-catalyzed kinetic resolution of the resulting *rac*-1-phenylethanol in $scCO_2$ (see Fig. 9A).[65] The use of cross-linked enzyme aggregates (CLEAs),[60] a carrier-free immobilized enzyme derivative obtained from the cross-linking of enzyme molecules by bifunctional reagents, demonstrated to be a superior biocatalyst than the classical immobilized enzyme derivative (Novozym 435). This work clearly shows how performing reactions in series displays a considerable advantage over performing the reactions separately, in particular related to the absence of SCF depressurization between reactions. Therefore, the economic productivity of the overall process was increased when a metal-catalyzed reaction was combined with a selective biocatalytic reaction in a multiple-step synthesis in $scCO_2$.

The use of cells as biocatalysts in continuous flow processes involving $scCO_2$ has also been reported. An example is the use of immobilized resting cells of *G. candidum* for the continuous reduction of cyclohexanone under supercritical conditions (see Fig. 9B).[66] The biocatalyst was recycled up to four times with only a slight loss in activity. Recycling was not possible using the corresponding batch system because biocatalysts did not tolerate both repeated depressurization at a very low temperature and separation of the product from the biocatalysts using organic solvents. This method was also applied for the asymmetric reduction of *o*-fluoroacetophenone, achieving excellent enantioselectivity (ee >99%) and a higher space-time yield than the corresponding batch process (0.24 µmol.min^{-1} *vs*. 0.13 µmol.min^{-1}, at 35°C and 100 bar).

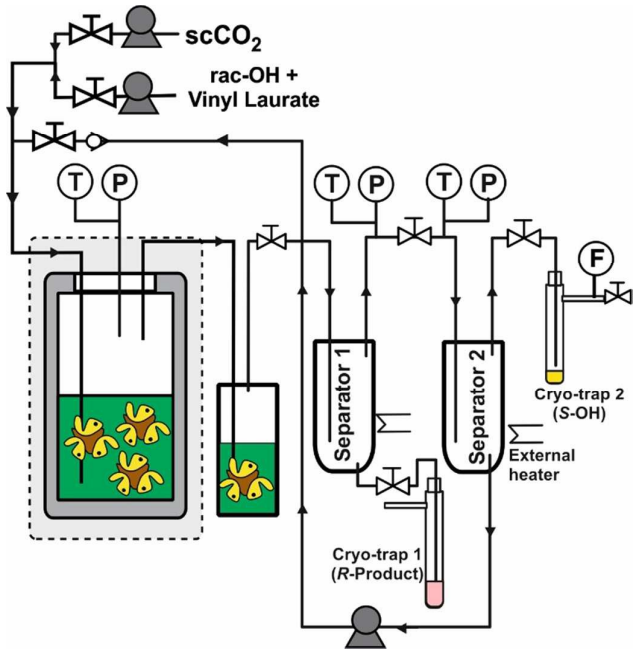

Fig. 10. Set-up of a reaction/separation system for continuous-flow combination of enzymatic kinetic resolution and enantiomer separation using an ionic liquid/$scCO_2$ medium]. P, pressure control; T, temperature control; F, Flow control).[74]

5. Integrated flow processes in neoteric solvents.

The combination of both ILs and $scCO_2$ neoteric solvents results in biphasic systems with several positive synergies, headed by the stabilization of biocatalysts, and it has opened up new opportunities for developing integral green processes in non-aqueous environments.[4a,5,20,67] The pioneering work of Brennecke's group demonstrating that ILs (*e.g.* [Bmim][PF_6]) and $scCO_2$ form biphasic systems was crucial.[68] Thus, the high solubility of $scCO_2$ in the IL phase, being able to extract previously dissolved hydrophobic compounds (*e.g.* naphthalene), was a useful opportunity to separate $scCO_2$-soluble products from reaction media based on ILs.[69] This discovery was key for further developments in multiphase green catalytic processes involving both chemical transformation and product extraction steps. Catalysis in multiphase operation offers promising opportunities for developing continuous chemical processes (*e.g.* the catalyst operates in one phase and the product is continuously delivered and extracted in the second phase).[4a,5,55,70]

Biocatalysis in IL/$scCO_2$ biphasic systems was originally described in 2002, as the first operational approach for developing fully green chemical processes in non-aqueous environments.[71] By this approach, the $scCO_2$ flow transports the substrate(s) to the IL phase containing the biocatalyst, and extracts the product(s) from the IL phase afterwards. Subsequently, the product(s) are obtained IL-free from the reactor by simple decompression, whereas CO_2 can be recycled by re-compression. Additionally, if the reaction product does not require any further purification, the approach enhances the economic benefit of the process, because the system runs as a black-box able to transform pure substrates into pure products without waste generation. Biotransformations occur into an IL phase (catalytic phase), while substrates and products remain largely in the SCF phase (extractive phase). It should be noted that an appropriate knowledge of the phase behaviour of the IL/$scCO_2$ systems is essential for developing any process, because it determines the contact conditions between $scCO_2$ and solutes. This includes the partitioning behaviour of organic compounds between both neoteric solvents, as well as the conditions for reducing the viscosity of the IL phase, thus enhancing the mass-transfer rate of any catalytic system.[67]

This green biocatalytic approach was firstly tested for two different reactions catalysed by CALB: aliphatic esters synthesis by transesterification between 1-alkanols and vinyl esters (*e.g.* butyl butyrate from vinyl butyrate and 1-butanol), and the kinetic resolution of *rac*-1-phenylethanol in a wide range of conditions (100-150 bar and 40-100ºC).[71a] Under these conditions, the enzyme showed an exceptional level of activity, enantioselectivity (*ee*> 99.9) and operational stability (*e.g.* the enzyme only lost 15% activity after 11 cycles of 4 h). Thus, excellent results can also be obtained for biotransformations in $scCO_2$ using the enzyme coated with ILs even under extreme conditions, *i.e.* 100 bar and 150ºC.[72] By the appropriate selection of the IL with respect to the polarity features of substrates and products, clear improvements of productivity can be obtained because of the better mass-transfer phenomena between IL and $scCO_2$ phases.[73]

A further step towards green biocatalysis in IL/$scCO_2$ biphasic systems was the appropriate selection of the acyl donor in the CALB-catalysed kinetic resolution (KR) of *rac*-1-phenylethanol, because the selective separation of the synthetic product can be included as an integrated step in the full process. By increasing the alkyl chain length of the acyl donor (*i.e.* vinyl laurate), the stereoselective synthetic product (*R*-1-phenylethyl laurate) can be selectively separated from the non-reacted *S*-1-phenylethanol with $scCO_2$ into two different cryo-traps (see Fig. 10). This process takes advantage of the fact that the solubility of a compound in $scCO_2$ depends on both its polarity and its vapour pressure. Thus, if the alkyl chain of an ester product is long enough, its low volatility should mean that it is less soluble in $scCO_2$ than the corresponding alcohol. Using this experimental approach, the introduction of two additional separation chambers connected with cryo-traps, and the selection of an appropriate pressure and temperature, resulted in the selective separation of the synthetic product and the unreacted alcohol from the reaction mixture (66% yield, ee>99.9%).[74]

Integrated multicatalytic processes, whereby one initial substrate is catalytically transformed into one final product by two or more consecutive catalytic steps in the same reaction system, is of great interest for developing future chemical industry.[5,64] The dynamic kinetic resolution (DKR) of *sec*-alcohols can be taken as an example to illustrate a clean

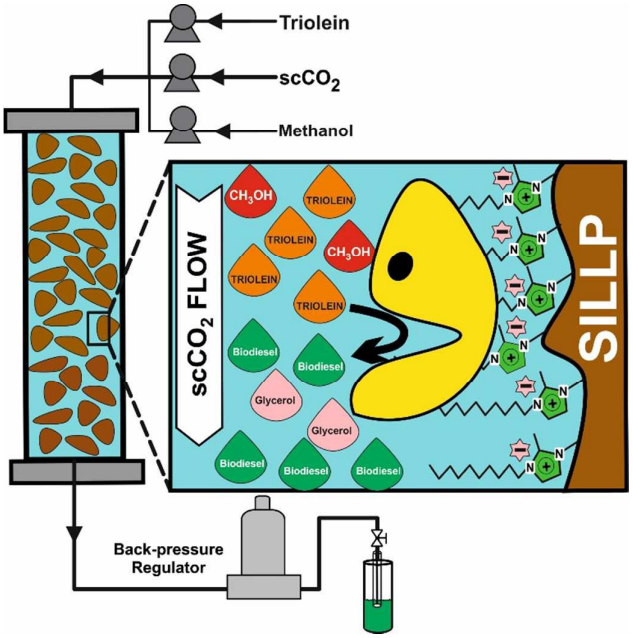

Fig. 11. Continuous flow reactor based on CALB immobilised on covalently supported ionic liquid-like phases (SILLPs) for the synthesis of biodiesel in $scCO_2$.[84]

multicatalytic approach in IL/$scCO_2$ biphasic systems. A DKR process is based on the combination of an enzymatic kinetic resolution (KR) reaction with an *in situ* chemical racemisation of the undesired enantiomer, which theoretically should permit to reach up to 100% of one enantiomeric pure product. This approach was successfully demonstrated by combining an immobilized CALB with a faujasite-type zeolite (CBV400) into a packed bed reactor under $scCO_2$ at 50ºC and 100 bar. By coating both chemical and enzymatic catalysts with [Btma][NTf_2], a nearly pure enantiomeric product (98% yield, 96% *ee*) was directly obtained at the outlet of the reactor for 14 days of continuous operation and without any loss in enzyme activity.[75] This work clearly shows the exciting potential of multi-catalytic (enzymatic or chemo-enzymatic) systems in ILs/$scCO_2$ for synthesizing optically active pharmaceutical drugs by a sustainable approach.

Another interesting example is the chemoenzymatic cascade oxidation in $scCO_2$–water biphasic media for the enantioselective sulfoxidation of thioanisole.[76a] In this system, Pd(0) catalyses the formation of H_2O_2 from H_2 and O_2 in the supercritical phase; the peroxide is subsequently used by the chloroperoxidase as an oxidant for the asymmetric sulfoxidation in the aqueous phase. In spite of the moderate reaction yields (14–60%), and the significant activity loss of the enzyme with time (up to 90% in 3 days at 40ºC and 130 bar), this work exemplifies the potential of compartmentalisation of the catalytic processes in multiphase systems. The alkene epoxidation of olefins with enzymatically generated peracids in hydrogen-bond-donating ILs (*e.g.* 1-(3-hydroxypropyl)-3-methylimidazolium nitrate) is another example of a chemo enzymatic process carried out using a lipase-catalysed cascade and hydrogen peroxide as the terminal oxidant (i.e. up to 86% yield for 24 h reaction).[76b]

A further step towards optimised IL/$scCO_2$ biphasic systems arose from the development of the immobilization of the ILs species onto solid supports. The immobilisation facilitates the separation processes and avoids a possible accidental spill on the environment. Besides, it reduces the process cost, as a minimal amount of IL is employed in catalytic processes in those biphasic systems.[77] The supported ionic species can be obtained either by adsorbing ILs onto solid supports (*Supported Ionic Liquid Phases* or *SILPs*),[4a,71-73] or by covalently binding IL-like fragments on the surface of the solid support (*Supported Ionic Liquid-like Phases* or *SILLPs*).[5,78]
The development of covalently Supported Ionic Liquid-Like Phase (SILLP) either by functionalization of PS-DVB surfaces with IL-like (imidazolium) moieties or by polymerisation of the corresponding functional monomers has open a new way to greatly reduce the amount of ILs and to facilitate their full reuse/recovery in continuous green chemical processes. In this approach, ILs properties are transferred onto the solid phase leading to a supported ionic liquid-like phase (SILLP), either in particles or monoliths.[79] A large diversity of SILLPs, varying cation, anion, as well as support nature and loading, have been characterized, including their thermal stability and polarity, which was dependent on the polarity induced by structural changes in the IL-like moieties. Thus, the SILLPs might be regarded as "solid solvents" or as nanostructured materials with microenvironments of tuneable polarity able to immobilised and stabilise catalytic species.[5]

These SILLPs have been successfully used as supports for metal catalysts,[80] organocatalysts[81] and biocatalysis.[15b] Bioreactors based on SILLPs were prepared as styrene-divinylbenzene polymeric monoliths containing imidazolium units (*ca*. 55 to 40% wt IL per gram of polymer), resulting in a IL-coating of the solid support surface. These SILLPs were able to adsorb *C. antarctica* lipase B, leading to highly efficient and robust heterogeneous biocatalysts, as it was observed for the flow synthesis of citronellyl propionate in $scCO_2$ (100 bar and 40-100ºC).[82] The catalytic activity of these mini-flow-bioreactors remained nearly unchanged for seven operational cycles of 5 h each in different supercritical conditions. This excellent catalytic efficiency of CALB-SILLPs derivatives was also observed for the DKR of *rac*-1-phenylethanol by using a single reactor in continuous operation under supercritical conditions. In this reactor, both the immobilized enzyme and an acid catalyst (*e.g*. zeolite CP811E-150) were mixed together to perform a "one-pot" catalytic system in $scCO_2$. By using this approach, the CALB-SILLPs derivatives did not require any additional coating with an IL layer to achieve good activity and stability. The optimisation of the experimental conditions (flow, pressure, temperature, etc.) increased the yields of the desired product up to 92% with *e.e*. >99.9% for the continuous DKR of *rac*-phenylethanol with vinyl propionate in $scCO_2$.[83] In another example, different nanostructured supports, based on different 1-decyl-2-methy-imidazolium cations covalently attached onto a polystyrene-divinylbenzene porous matrix (SILLPs), were used as carriers to immobilize CALB. The

suitability of these immobilized lipase derivatives to carry out the synthesis of biodiesel (methyl oleate) by methanolysis of triolein has been tested in both *t*-butanol and $scCO_2$ (180 bar, 45 ºC) as reaction media (see Fig. 11). The use of modified supports with low IL loading covalently attached to the main polymeric backbone chains provide structured materials leading to the best biodiesel yields (up to 95%) and operational stability (85% biodiesel yield after 45 cycles of 8-4 h) in $scCO_2$ (45 ºC, 180 bar). The presence of *t*-butanol, as an inert cosolvent, in the $scCO_2$ phase was key to avoid the poisoning of the biocatalyst by the polar by-product glycerol.[84] These results clearly illustrate the potential of SILLPs-supported biocatalysts for the production of a green biofuel, as biodiesel, by a fully green technology under flow operation.

6. Applications of ionic liquids in DNA technology.

Nucleic acids are biopolymers characterized by their great conformational flexibility that permits their adaptation to the new media conditions or functional requirements. In this sense, the huge tunability of ILs can provide new solubilisation media for many applications, from DNA isolation to stabilization of conformational structures for technological applications.[85] In this context, the study of UV melting curves of DNA sequences has shown that A-T base pairs are more stable in the choline-dhp (hydrated choline dihydrogen phosphate, CDP) IL than G-C base pairs, as compared with water.[86] This IL also increases the stability of Hoogsteen interactions, making it comparable to that of Watson-Cricks base pairs, which seems to rely on the electrostatic interactions between the organic cations of the ILs-network with the phosphate backbone at the minor grooves in DNA, while anions mainly form hydrogen bonds with cytosine, adenine and guanine bases.[87] All these interactions are responsible of a highly stable structure of double stranded DNA (dsDNA) in ILs that retains the native B-form crystallographic structure. Additionally, as these ILs avoid the proper folding of proteins, *e.g.* nucleases, they help to reduce DNA degradation during storage.[86]

The higher stability of dsDNA and triplex DNA in ILs makes possible the use of probes that recognize a specific sequence without a previous step of denaturation. This simplifies the methodology required for sensing devices for particular DNA sequences, such as DNA microarrays, Southern blotting or *in situ* hibridation, of great relevance in medicine and nanobiosensing.[88] It should be highlighted the special relevancy of G-quadruplexes in biological systems, especially in cancer. For example, they are present in human telomeres, where they inhibit telomerase activity, and in oncogene promoter regions, where they regulate gene expression.[89] G-quadruplexes are highly polymorphic according to the media conditions or different stimuli, which has raised a great interest in non-biological applications as catalysts, biosensors and DNA-based architectures.[90] Since for many applications the conditions must be strictly anhydrous, deep eutectic solvents (DESs, which shares most of the remarkable qualities of ILs but differ from ILs in the presence of a non-ionic organic

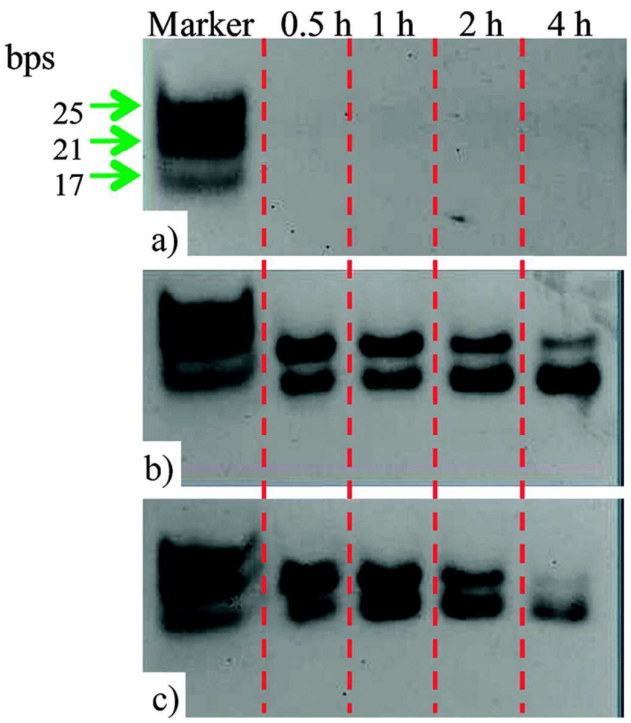

Fig. 12. Timed degradation profile of a siRNA in (a) phosphate buffer, (b) 20% (w/w) CDP IL and (c) 50% (w/w) CDP IL after incubating for specified time intervals in RNase A solution at 37ºC. *Reproduced from Ref. 94 with permission from the Royal Society of Chemistry*.

molecular component as predominant constituent) provide an excellent water free medium that stabilizes G-quadruplexes in their parallel favoured conformation.[91]

Related to this characteristic of molecular biosensing through structural changes in DNA sequences, aptamers are single stranded nucleic acids (ssDNA or RNA) that specifically recognize desired targets, such as proteins, amino acids, antibiotics or small molecules.[92] In this way, Machado *et al.* [93] have studied the influence of ethylammonium nitrate in the capacity of molecular recognition of an AMP aptamer molecular beacon. At high concentrations, this IL reduced the thermal stability of the intramolecular dsDNA structure of the probe, and accelerates the hybridization rate of ssDNA. Nevertheless, this exceptional characteristic of increased stability of nucleic acids in ILs is not only circumscribed to DNA. As can be seen in Fig 12, the hydrated CDP (buffered choline hydrogen phosphate) IL is able to prolong the shelf-life of small interfering RNA (siRNA) for up to three months, even in the presence of RNase A.[94] The IL prevents the de-winding of dsRNA, as well as its cleavage by RNase A at 37ºC incubation. The use of ILs as solvents offers an extraordinary chance for siRNA easy handling, which is the main problem in the strategy of silencing gene expression.

Compaction is also an important issue in long-term storage DNA, because of the increased resistance against chemical, biochemical and mechanical stress, like UV radiation.[95] In this way, it has been also reported how guanidinium

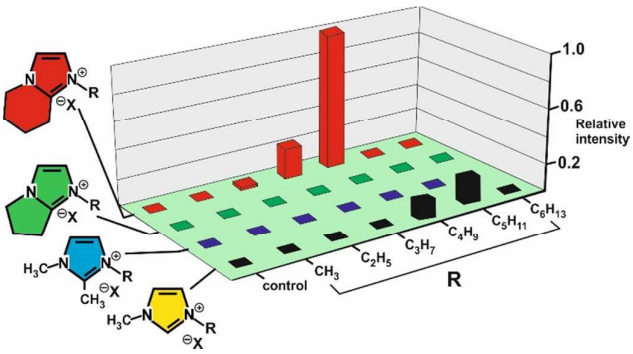

Fig. 13. Screening of the suitability of ILs (70 mM) to promote PCR amplification of a GC-rich (80% GC content), 266 bp gene fragment. *Reproduced from Ref. 102 with permission from the Royal Society of Chemistry*.

tris(pentafluoroethyl)trifluorophosphate IL (Gua-IL) can increase intra- and interstrand attractions between DNA molecules by compensating DNA backbone charges through electrostatic interactions.[96] The Gua-IL induced compaction is comparable to that induced by histone proteins, nanoparticles, dendrimers and PEG.

The excellent suitability of ILs for dissolving and stabilizing DNAs has opened the door to their testing in the isolation and purification of DNA from aqueous solutions.[97] DNA extraction represents a significant bottleneck in many applications due to the complexity of isolating highly pure DNA from a cellular matrix or complex environmental samples. The need of eliminating contaminants like proteins, small organic molecules, polysaccharides, phospholipids as well as organic solvents that may interfere with downstream applications, have encouraged the development of new methodologies with high throughput, purity and recovery. One of the first attempts was the use of ILs for *in situ* dispersive liquid-liquid microextraction of DNA.[98] This method showed a high extraction efficiency of DNA, although the DNA recovery from the IL solvent was non-efficient. Since then, several approaches have been carried out, such as Fe_3O_4 magnetic nanoparticles (NPs) modified with [C_6MIM][Br] (1-hexyl-3-methylimidazolium bromide), which improves the rate of DNA adsorption and subsequent stripping for their recycling.[99] In the same context, a fast method for plasmid DNA extraction based on polymeric IL microspheres (PIL, poly [1-vinyl-3-(2-methoxy-2-oxylethyl)imidazolium] [PF_6]) has been reported.[100] This PIL exhibits an excellent capacity of DNA adsorption/extraction (up to 190.7µg DNA/mg PIL) with respect to other methods described to date, as well as an easy stripping from the microspheres by simply regulating the salt concentration. Even more, the application of ILs in DNA extraction has been improved by introducing hydrophobic magnetic ILs solvents that combine the tunability of the IL with the magnetic nature of the solvent.[101] The use of these magnetic ILs allows avoiding the centrifugation steps, which are time-consuming and may affect the quality of the isolated DNA.

Furthermore, it should be highlighted how the role of ILs come out as a key to solve serious technical problems in Molecular Biology, such as the poor amplification of GC-rich DNA sequences by PCR.[102] These sequences are very common in promoter elements that regulate gene expression, and there is an obstacle for several purposes where PCR is routinely used, such as sequencing and replication of genes, detection and diagnosis of diseases, forensic identification of genetic fingerprints or manipulation of transgenic organisms. Thus, it has been demonstrated that a bicyclic imidazolium IL is highly effective not only for promoting PCR of GC-rich DNA by minimizing non-specific amplification, but also for facilitating PCR of normal-GC DNA under mild conditions (see Fig. 13).[102] Finally, it should also be pointed out the potential of applications of ILs in the field of nanobiosensing. In this way, deserve to the mentioned here the reports on the combination of ILs with nanomaterials (*e.g.* CeO_2-SWNTs-BMIMPF_6, etc.) for the hybridization and detection of DNA,[103] the development of DNA films with high ionic conductivity by using ILs,[104] as well as the carbon IL composite electrodes (CILE) based on chitosan/Fe_3O_4 microsphere-graphene carriers to immobilize ssDNA probes.[105] These electrodes show a high rate of recognition, good stability and high grade of discrimination between DNA sequences, as reported for the product of soybean lectin gene sequence.[106] The exceptional qualities of ILs for nucleic acids technologies make them suitable for a wide range of applications in medicine and nanotechnology in the near future.

6. Conclusions

Nature has always been a source of inspiration for chemists. The design of multicatalytic processes, mimicking metabolic pathways, to achieve a maximum catalytic efficiency towards desired products with the minimum production of wastes, is the main goal of sustainable chemistry. For this aim, nature provides us active biopolymers (i.e. enzymes and nucleic acids), which are the most powerful toolbox for carrying out selective, clean and sustainable transformations, as well as for developing new technologies for molecular footprinting analysis in medicine, food, etc. The XXI century is the century of sustainability, where the development of a network of green chemical processes will depend on the implementation of new integrated reaction / separation systems, where active biopolymers are the core.

The use of biocatalytic approaches in green non-conventional reaction media holds much promise for the development of a sustainable chemical manufacturing industry. Biocatalytic processes in non-aqueous environments enhance the possible technological applications and expand the repertoire of enzyme mediated transformations. Furthermore, it has been demonstrated that ILs have an exceptional ability to over-stabilize enzymes, or to provide exceptional properties to nucleic acids, opening a new window of processing options not available using conventional organic

solvents. The excellences of hydrophobic ILs for enzyme catalysis have been supplemented by the phase behaviour of ILs based on cations with long alkyl chains, also named Sponge-Like Ionic Liquids (SLILs). The sponge-like character of these ILs allows the development of easy and green methods for efficiently separating a hydrophobic organic compound (*e.g*. biodiesel) from a homogeneous ionic liquid/organic compound mixture using a simple cooling and centrifugation approach, as opposed to the usual heating step applied in classical separation processes in Chemical Engineering (e.g. distillation).

On the same way, SCFs are being increasingly used to carry out enzymatic reactions. SCFs improves enzymatic reaction rates, simplify product recovery, and are ideal replacement for conventional organic solvents as they are more environmentally friendly. Furthermore, the unique phase behaviour of IL/scCO_2 systems allows processes involving reaction and downstream isolation and purification steps, facilitating the easy reuse of the catalyst and the IL phase. The combination of enzymes with multiphase neoteric systems should be explored as a clear strategy for developing integral new green synthetic processes. Multi-enzymatic and/or multi-chemoenzymatic green chemical processes in multiphase neoteric systems for synthesizing pharmaceutical drugs are only a starting point, but demonstrated that the development of a sustainable chemical industry is possible and it should be irreversible.

Acknowledgements

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