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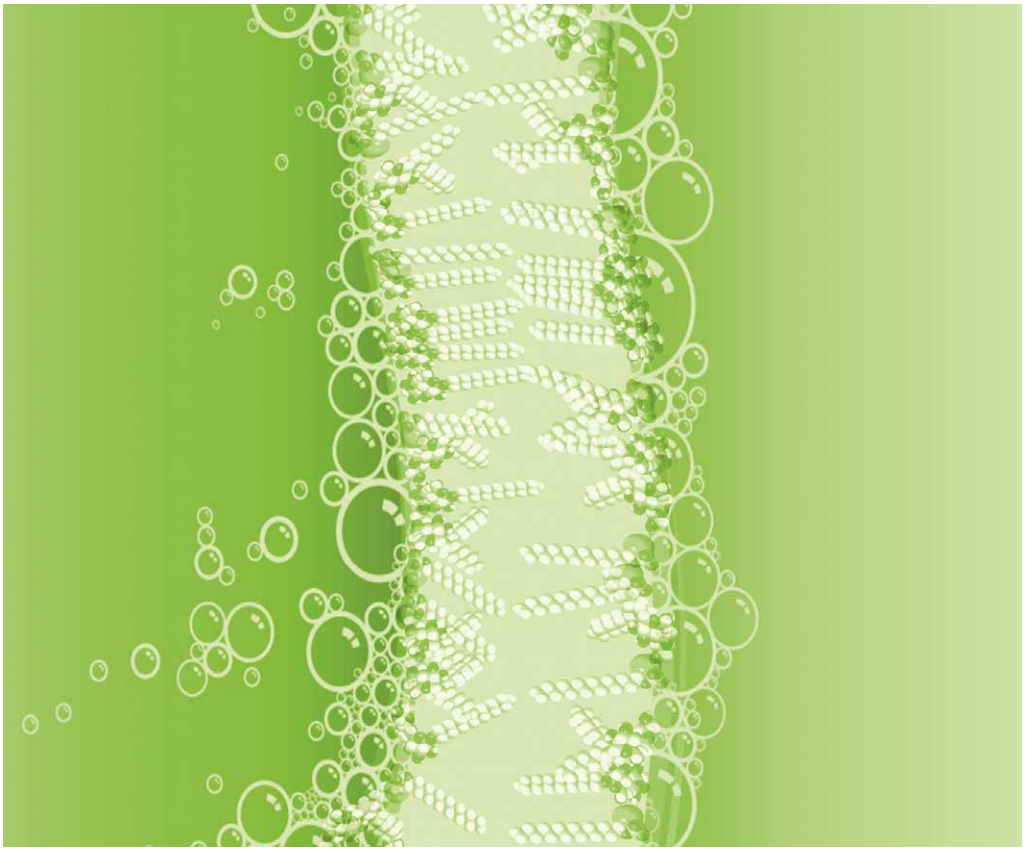

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TUTORIAL REVIEW

Fundamentals of green chemistry: efficiency in reaction design†‡

Roger A. Sheldon

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In this *tutorial review*, the fundamental concepts underlying the principles of green and sustainable chemistry - atom and step economy and the E factor - are presented, within the general context of efficiency in organic synthesis. The importance of waste minimisation through the widespread application of catalysis in all its forms – homogeneous, heterogeneous, organocatalysis and biocatalysis – is discussed. These general principles are illustrated with simple practical examples, such as alcohol oxidation and carbonylation and the asymmetric reduction of ketones. The latter reaction is exemplified by a three enzyme process for the production of a key intermediate in the synthesis of the cholesterol lowering agent, atorvastatin. The immobilisation of enzymes as cross-linked enzyme aggregates (CLEAs) as a means of optimizing operational performance is presented. The use of immobilised enzymes in catalytic cascade processes is illustrated with a trienzymatic process for the conversion of benzaldehyde to (*S*)-mandelic acid using a combi-CLEA containing three enzymes. Finally, the transition from fossil-based chemicals manufacture to a more sustainable biomass-based production is discussed.

1. Introduction: Efficiency in organic synthesis

The disciplines of organic chemistry and catalysis date back to Berzelius who coined both terms, in 1807 and 1835, respectively.[1] Berzelius was a staunch believer in vitalism which held that organic substances derived from living matter are endowed with a mystical 'vital force' (*vis vitalis*) which precudes their synthesis in the laboratory. Hence, Wöhler's synthesis of the natural product urea from ammonia and cyanic acid in 1828 had monumental significance. It clearly showed that, in principle, organic compounds are amenable to synthesis in the laboratory and heralded the demise of the vital force theory.

Another landmark in the development of organic synthesis was the serendipitous preparation of the first synthetic dye, mauveine (aniline purple) by Perkin in 1856, regarded by many as the first industrial organic synthesis.[2] Ironically, Perkin's goal was the synthesis of the anti-malarial drug, quinine, by oxidation of N-allyl toluidine with potassium dichromate. This discovery marked the advent of the synthetic dyestuffs industry from coal tar, a byproduct of steel manufacture. It was quickly followed by efficient syntheses of many natural dyes, the commercialization of which signalled the demise of their production from renewable raw materials.

The modern pharmaceutical and fine chemical industries evolved as spin-offs of the synthetic dyestuffs industry. The first synthetic drugs were relatively simple molecules but in the ensuing decades they became increasingly complicated, as exemplified by the introduction of the semi-synthetic beta-lactam antibiotics and the steroid hormones in the 1940s and the anti-cancer drug, taxol, in the 1990s. Indeed, the success of the modern pharmaceutical industry is largely due to the remarkable achievements of organic synthesis over the last century. However, many of these time-honored and widely applicable reactions were developed at a time when the toxic properties of many reagents and solvents were not known and waste minimisation and sustainability were not significant issues.

Department of Biotechnology, Delft University of Technology, Julianalaan 136, Delft 2628BL, Netherlands.
E-mail: r.a.sheldon@tudelft.nl; Fax: +31 (0)15 2781415; Tel: +31 (0)15 2782675

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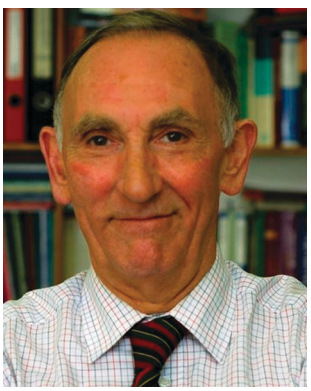

Roger A. Sheldon

Roger Sheldon is Professor emeritus of Biocatalysis and Organic Chemistry at Delft University of Technology and Chief Executive Officer of CLEATechnologies B.V. He has a PhD (1967) from the University of Leicester for research on organophosphorus chemistry under the joint supervision of Professors Stewart Trippett and Stephen Davidson. From 1967 to 1969 he was a postdoctoral fellow in the group of Professor Jay Kochi at Case Western Reserve University (Cleveland) and Indiana University. Prior to his appointment in Delft, he was with Shell Research Laboratories in Amsterdam (1969-1980) and was vice president for R&D at DSM Andeno (1980–1991).

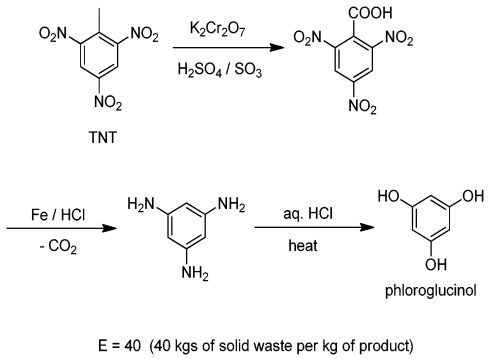

Fig. 1 A process for the production of phloroglucinol.

Some of them are essentially the same as those used by Perkin and contempories almost two centuries ago. For example, up until the mid-1980s, the reprographic chemical and pharmaceutical intermediate, phloroglucinol (1,3,5-benzene triol), was produced mainly from 2,4,6-trinitrotoluene (TNT) by the process shown in Fig. 1.[3] The first step involves oxidation with potassium dichromate, the very same reagent used by Perkin in 1856. What can we say about the efficiency of this process? It produces phloroglucinol in >90% overall yield over three steps and, according to classical concepts of reaction efficiency, would generally be considered an efficient process. But is it really so efficient? In addition to the desired product, the process generates 40 kgs of solid waste, containing $Cr_2(SO_4)_3$, NH_4Cl, $FeCl_2$ and $KHSO_4$, for every kg of phloroglucinol formed. It was eventually discontinued because of the prohibitive costs associated with the disposal of this chromium-containing waste.

The phloroglucinol amounts to only 5% of the total mass of products formed in the stoichiometric equation (see Fig. 2), the remainder comprising mainly inorganic salts. The reaction stoichiometry predicts the formation of *ca.* 20kgs of waste per kg of phloroglucinol. However, this corresponds to the ideal situation where exactly stoichiometric amounts of reagents are used and the chemical yield is 100%. In practice, an excess of the oxidant and reductant, and a large excess of sulfuric acid, which has to be subsequently neutralized with base, is used and the isolated yield of phloroglucinol is *ca.* 90%. This readily explains the observed formation of 40 kgs of waste per kg of desired product, rather than the *ca.* 20kgs that would be expected on the basis of the stoichiometric equation.

The sheer magnitude of waste generation in this process was an eye opener and a subsequent analysis of the waste formed in processes for the manufacture of pharmaceuticals and fine and even bulk chemicals revealed that it was by no means an exception in these industries. It subsequently formed the basis for the development of the E factor concept (see later) for

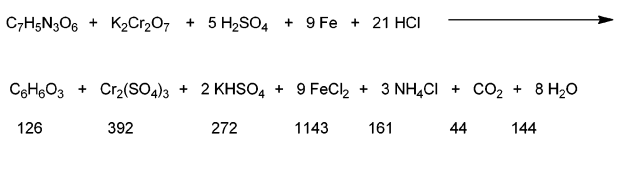

Fig. 2 The stoichiometry of the phloroglucinol process.

assessing the environmental impact of chemical manufacturing processes. It became clear that a paradigm shift was needed in industrial organic synthesis from traditional concepts of reaction efficiency and selectivity, that focus largely on chemical yield, to one that assigns value to raw materials utilisation, elimination of waste and circumventing the use of toxic and/or hazardous substances.

Synthetic organic chemists were well-acquainted with terms such as reaction selectivity (yield divided by conversion) and chemo-, regio-, stereo- and enantio-selectivity. In contrast, prior to the mid 1990's, they were not accustomed to considering what we called the atom selectivity or atom utilisation of a reaction, *i.e.* how much of the mass of the reactants actually ends up in the product, the rest being, by definition, waste. This selectivity is of the utmost importance in the context of assessing the environmental impact of organic syntheses on an industrial scale.

2. Sustainability and green chemistry

The World Commission for Environment and Development, founded in 1983 by the United Nations, was given the task of preparing a report on the perspectives of long-term, sustainable and environmentally friendly development on a world scale by 2000 and after. This culminated in the publication, four years later, of the report, *Our Common Future*,[4] also known as the *Brundtland Report* after the Prime Minister of Norway who was Chairman of the commission at that time. The report defined sustainable development as:

Meeting the needs of the present generation without compromising the ability of future generations to meet their own needs.

In the succeeding two decades the concept of sustainability has become the focus of considerable attention both in industry and in society as a whole.

The term 'Green Chemistry' was coined in the early 1990s by Anastas and colleagues[5] of the US Environmental Protection Agency (EPA). In 1993 the EPA officially adopted the name 'US Green Chemistry Program' which has served as a focal point for activities within the United States, such as the Presidential Green Chemistry Challenge Awards and the annual Green Chemistry and Engineering Conference. This does not mean that research on green chemistry did not exist before the early 1990s, merely that it did not have the name. The guiding principle is *benign by design* of both products and processes.[6] This concept is embodied in the 12 Principles of Green Chemistry which can be paraphrased as:

1. Waste prevention instead of remediation
2. Atom efficiency
3. Less hazardous/toxic chemicals
4. Safer products by design
5. Innocuous solvents and auxiliaries
6. Energy efficient by design
7. Preferably renewable raw materials
8. Shorter syntheses (avoid derivatization)
9. Catalytic rather than stoichiometric reagents
10. Design products for degradation
11. Analytical methodologies for pollution prevention
12. Inherently safer processes

Subsequently, Anastas and Zimmerman[7] proposed the twelve principles of green engineering which embody the same underlying

features – conserve energy and resources and avoid waste and hazardous materials – as those of green chemistry, but from an engineering viewpoint. More recently, a mnemonic, PRODUCTIVELY, was proposed by Poliakoff *et al.*[8] which captures the spirit of the twelve principles of green chemistry:

P – Prevent wastes
R – Renewable materials
O – Omit derivatisation steps
D – Degradable chemical products
U – Use of safe synthetic methods
C – Catalytic reagents
T – Temperature, Pressure ambient
I – In-Process monitoring
V – Very few auxiliary substrates
E – E-factor, maximise feed in product
L – Low toxicity of chemical products
Y – Yes, it is safe

Alternatively, the essence of green chemistry can be reduced to a working definition in a single sentence.[3]

Green chemistry efficiently utilises (preferably renewable) raw materials, eliminates waste and avoids the use of toxic and/or hazardous reagents and solvents in the manufacture and application of chemical products.

Raw materials include the source of the energy used in the process as this leads to waste in the form of carbon dioxide emissions. Green chemistry eliminates waste at source, *i.e.* it is primary pollution prevention rather than waste remediation (end-of-pipe solutions), as described by the first principle of green chemistry: prevention is better than cure. In the last fifteen years the concept of green chemistry has been widely embraced in both industrial and academic circles. One could say that sustainability is our ultimate common goal and green chemistry is a means to achieving it.

3. Green chemistry metrics: atom economy and the (E)nvironmental factor

Having defined what Green Chemistry is we need to be able to compare processes (and products) on the basis of their greenness. There is no absolute greenness, one process is greener than another process, but appropriate green metrics are a prerequisite for a meaningful comparison of greenness. To quote Lord Kelvin: "to measure is to know".

The most widely accepted measures of the environmental impact of chemical processes are, probably not coincidentally, the two most simple metrics: the *E factor*,[9–11] defined as the mass ratio of waste to desired product and the *atom economy*,[12] defined as the molecular weight of the desired product divided by the sum of the molecular weights of all substances produced in the stoichiometric equation, expressed as a percentage.

A knowledge of the stoichiometric equation allows one to predict, without performing any experiments, the theoretical amount of waste that can be expected. Our experience with the phloroglucinol process (see above) led us to use what we called *atom utilisation* to quickly assess the environmental acceptability of alternative processes to a particular chemical.[13] It was a logical elaboration of the concepts of *syn gas utilisation*[14] and *oxygen availability* in different oxidants.[15] However, *atom economy (AE)*, introduced by Trost in 1991[15] has become the

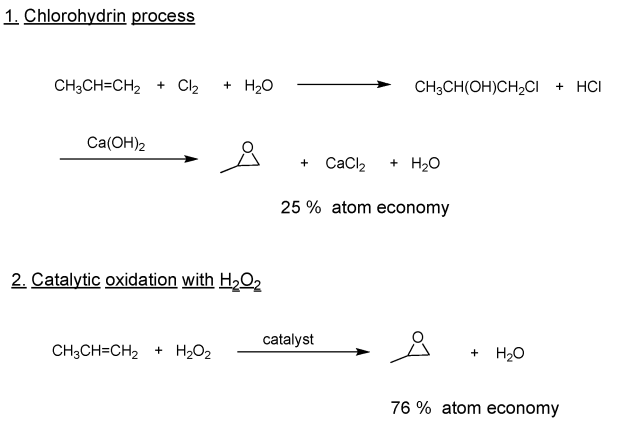

Fig. 3 Atom efficiencies of two processes for propylene oxide.

widely accepted terminology although *atom efficiency* (also abbreviated as AE and more in line with the title of this tutorial) is also used. In Fig. 3 we compare the AE of the classical chlorohydrin route to propylene oxide with that of oxidation with the green oxidant hydrogen peroxide, where the coproduct is water.[16] Purely on a weight basis, the former is actually a process to make calcium chloride with propylene oxide as the coproduct.

Atom economy is a theoretical number that is based on a chemical yield of 100% of theoretical and assumes that reactants are used in exactly stoichiometric amounts. It disregards substances, such as solvent and acids or bases used in work-up, which do not appear in the stoichiometric equation.

The E factor, in contrast, is the actual amount of waste produced in the process, defined as everything but the desired product. It takes the chemical yield into account and includes all reagents, solvents losses, all process aids and, in principle, even the energy required as this generates waste in the form of carbon dioxide. We generally excluded water from the calculation of the E factor as we surmised that its inclusion would lead to exceptionally high E factors in many cases and make meaningful comparisons of processes difficult. For example, when considering an aqueous waste stream only the inorganic salts and organic compounds contained in the water are counted while the water is excluded However, there is a definite trend, at least in the pharmaceutical industry, towards the inclusion of water in the E factor and we note that water usage can be a crucial issue in biomass conversion and in fermentation processes in general (see later).

A higher E factor means more waste and, consequently, greater negative environmental impact. The ideal E factor is zero. Put quite simply, it is kilograms (of raw materials) in, minus kilograms of desired product, divided by kilograms of product out. It can be easily calculated from a knowledge of the number of tons of raw materials purchased and the number of tons of product sold, for a particular product or a production site or even a whole company. We note that this method of calculation will automatically exclude water used in the process, but not water formed. The sheer magnitude of the waste problem in chemicals manufacture is readily apparent from a consideration of typical E factors, in various segments of the chemical industry, shown in Table 1, which we published almost two decades ago.

Table 1 E factors in the chemical industry

Industry Segment	Volume (tons/annum)[a]	E Factor (kg waste/kg product)
Bulk Chemicals	10^4–10^6	<1–5
Fine chemicals	10^2–10^4	5– >50
Pharmaceutical Industry	10–10^3	25– >100

[a] Annual production world-wide or at a single site.

It is also clear that the E factor increases substantially on going downstream from bulk chemicals to fine chemicals and pharmaceuticals. Indeed, when we began an inventory of the E factors of fine chemicals manufacture back in the late 1980s, it was clear that tens of kgs of waste per kg of product were more the norm than the exception and in pharma they were even higher. On the one hand, it is a direct consequence of the more widespread use of stoichiometric reagents in these industry segments. In contrast, in bulk chemicals manufacture the production volumes are enormous and the use of many stoichiometric reagents is, for purely economic reasons, generally prohibitive. On the other hand, it is a consequence of the fact that the target pharmaceuticals, for example, are more complicated molecules compared to bulk chemicals and, hence, their production involves multi-step syntheses which can be expected to generate more waste. Consequently, waste generation can be reduced by developing processes that are more step economic as promulgated by Wender.[17]

The E factor has been widely adopted by the chemical industry and in particular by the pharmaceutical industry,[18] as a useful barometer for assessing the environmental impact of manufacturing processes.[19,20]

Other metrics have been proposed for measuring the environmental acceptability of processes.[21–23] They can be categorized in two types: (i) metrics that constitute a refinement of the AE concept, *i.e.* those based on the stoichiometric equation of the reaction concerned, and (ii) metrics that are variations on the theme of the E factor, *i.e.* they address the actual amount of waste formed in the process. As examples of the former, Constable and coworkers[24] at Glaxo Smith Kline (GSK) introduced the terms *reaction mass efficiency* (RME) and *carbon efficiency* (CE). RME is defined as the mass of product obtained divided by total mass of reactants in the stoichiometric equation expressed as a percentage. It is a refinement of AE that takes the chemical yield of the product and the actual quantities of reactants used into account. A disadvantage compared to AE is that RME cannot be used for a quick analysis of different processes before any experimental work is performed. CE is similar to RME but takes only carbon into account, *i.e.* it is the mass of carbon in the product obtained divided by the total mass of carbon present in the reactants.

An example of the second category is *mass intensity* (MI) which was proposed by the GSK group.[25] It is defined as the total mass of materials used in a process divided by the mass of product obtained expressed as a percentage, *i.e.* MI = E factor +1 and the ideal MI is 1 compared with zero for the E factor. The same authors also suggested the use of so-called *mass productivity* which is the reciprocal of the MI. Hudlicky and coworkers[26] proposed an analogous metric: the *effective mass yield* (EMY), defined as the mass of the desired product divided by the total mass of non-benign reactants used in its preparation. The EMY does not include so-called environmentally benign compounds, such as NaCl, acetic acid, *etc.* This is questionable as the environmental impact of such substances is not zero and is volume-dependent. Defining non-benign is difficult and arbitrary and it was concluded,[24] therefore, that EMY suffers from a lack of definitional clarity.

In our opinion none of these alternative metrics offers any particular advantage over atom economy and the E factor for assessing how wasteful a process is. The former is a quick tool that can be used before conducting any experiments and the latter is a measure of the total waste that is actually formed in practice. Thus, AE and the E factor are complementary green metrics. As noted above, the AE of the phloroglucinol process (see Fig. 2) is *ca.* 5%, which would predict an E factor of *ca.* 20 but, in practice, the E factor is 40. This is because the overall yield is not 100%, a molar excess of the various reactants is used, and the sulfuric acid (which is used in large excess) has to be neutralized with base in the work-up.

This example is by no means an exception. The large amounts of waste generated in processes for the manufacture of fine chemicals and pharmaceuticals, and even some bulk chemicals, consist primarily of inorganic salts, such as sodium chloride, sodium sulfate and ammonium sulfate formed in the reaction or in subsequent neutralization steps.

4. The nature of the waste

All of the metrics discussed above take only the mass of waste generated into account. However, the environmental impact of waste is not only determined by its amount but also by its nature. One kg of sodium chloride is obviously not equivalent to one kg of a chromium salt. In order to take this into account, we introduced[10] the term 'environmental quotient', EQ, obtained by multiplying the E factor with an arbitrarily assigned unfriendliness quotient, Q. For example, one could arbitrarily assign a Q value of 1 to NaCl and, say, 100–1000 to a heavy metal salt, such as chromium, depending on its toxicity, ease of recycling, *etc.* The magnitude of Q is obviously debatable and difficult to quantify but, importantly, 'quantitative assessment' of the environmental impact of chemical processes is, in principle, possible.[27] We also note that Q for a particular substance can be influenced by both the volume of production and the location of the production facilities. For example, the generation of 100–1000 tons per annum of sodium chloride is unlikely to present a problem but 10 000 tons per annum, in contrast, may already present a disposal problem, thus warranting an increase in Q. Ironically, when very large quantities of sodium chloride are generated the Q value could decrease again as recycling by electrolysis becomes a viable proposition, *e.g.* in propylene oxide manufacture *via* the chlorohydrin route (see earlier). Thus, generally speaking the Q value of a particular waste will be determined by its ease of disposal or recycling. In our experience, organic waste is, generally speaking, easier to dispose of than inorganic waste and this is important when we consider the green metrics of biocatalytic processes (see later).

Another approach to assessing the environmental impact and sustainability of both products and processes in general is *Life Cycle Assessment (LCA)*.[28] This involves the evaluation

of products and processes within defined domains, *e.g.* cradle–to-gate, cradle-to-grave and gate-to-gate, on the basis of quantifiable environmental impact indicators, such as energy usage, global warming, ozone depletion, acidification, eutrophication, smog formation, and ecotoxicity, in addition to waste generated. In essence, LCA is an integration of amounts of waste with quantifiable effects caused by the waste, such as global warming and smog formation and, hence, resembles EQ.

5. Catalysis and waste minimisation

Ironically, the waste generated in the manufacture of organic compounds consists primarily of inorganic salts. This is a direct consequence of the use of stoichiometric inorganic reagents in organic synthesis, particularly in fine chemicals and pharmaceuticals manufacture. Examples, which readily come to mind are stoichiometric reductions with metals (Na, Mg, Zn, Fe) and metal hydride reagents ($LiAlH_4$, $NaBH_4$), oxidations with permanganate, manganese dioxide and chromium(VI) reagents. A classic example is the phloroglucinol process discussed above, which combines an oxidation with stoichiometric amounts of chromium (VI) with a stoichiometric reduction with Fe/HCl. Similarly, a multitude of reactions, *e.g.* sulfonations, nitrations, halogenations, diazotisations and Friedel-Crafts acylations, employing stoichiometric amounts of mineral acids (H_2SO_4, HF, H_3PO_4) and Lewis acids ($AlCl_3$, $ZnCl_2$, BF_3) are major sources of waste.

The solution to the waste problem is self-evident: substitution of antiquated stoichiometric methodologies with green catalytic alternatives[10,11,12,29] that are more atom and step economic, and, consequently, have lower E factors. For example, catalytic hydrogenation, oxidation and carbonylation (Fig. 4) are highly atom efficient, low-salt processes. Traditionally, catalysts are divided into 4 sub-categories: heterogeneous, homogeneous, organocatalysts and biocatalalysts and examples of the various types will be treated in the ensuing discussion.

6. Solvents, multiphase catalysis and reaction efficiency

Another major source of waste is solvent losses, which end up in the atmosphere or in ground water. Indeed, solvent losses are a major contributor to the high E factors of pharmaceutical manufacturing processes.[30] Furthermore, health and/or safety issues associated with many traditional organic solvents have led to their use being severely curtailed. The FDA has issued guidelines for solvent use in the pharmaceutical industry (see www.fda.gov/cder/guidance/index.htm). Solvents are divided into four classes:

Class 1 solvents should not be used in the manufacture of drug substances because of their unacceptable toxicity or deleterious environmental effects. They include benzene and various chlorinated hydrocarbons.

Class 2 solvents should be used only sparingly in pharmaceutical processes because of inherent toxicity and include acetonitrile, dimethyl formamide, methanol and dichloromethane.

Class 3 solvents may be regarded as less toxic and of lower risk to human health. They include many lower alcohols, esters, ethers and ketones.

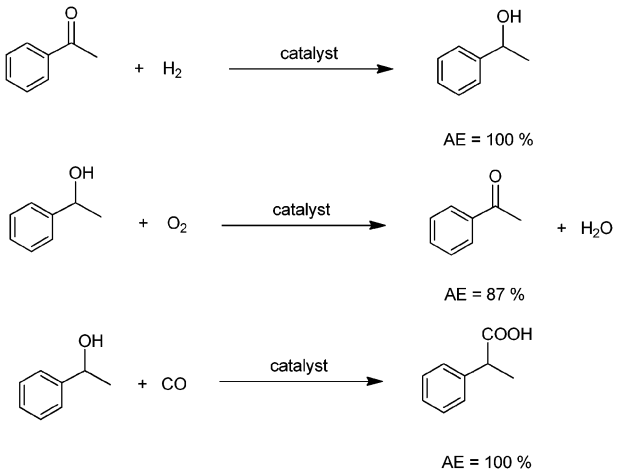

Fig. 4 Atom efficient processes.

Class 4 solvents, for which no adequate data are available, include di-isopropyl ether , methyl tetrahydrofuran and isooctane.

Many pharmaceutical companies are focusing their attention on minimising solvent use and in replacing many traditional organic solvents, such as chlorinated and aromatic hydrocarbons, by more environmentally friendly alternatives such as lower alcohols, esters and some ethers such as methyl tert butyl ether (MTBE). Pfizer scientists[18] for example, have produced a solvent selection guide for medicinal chemists, dividing solvents into three categories: undesirable (red), usable (yellow) and preferred (green) as shown in Fig. 5. Solvents derived from renewable feedstocks, such as ethanol and methyl tetrahydrofuran are becoming popular and ethyl lactate, produced by combining two innocuous renewables is currently being promoted as a reaction medium.

In our original inventory of E factors of various processes we assumed, in the absence of concrete data, that solvents would be recycled by distillation and that this would involve a 10% loss. However, organic chemists have a marked tendency for using different solvents for the various steps in multistep syntheses making recycling difficult owing to cross contamination. In the redesign of the sertraline manufacturing process,[31] for which Pfizer received a Presidential Green Chemistry Challenge Award in 2002, among other improvements a three step sequence was streamlined employing ethanol as the sole solvent, followed by a fourth step in ethyl acetate (Fig. 6). This eliminated the need to

Preferred	Usable	Undesirable
Water	Cyclohexane	Pentane
Acetone	Toluene	Hexane(s)
Ethanol	Methylcyclohexane	Di-isopropyl ether
2-Propanol	TBME	Diethyl ether
1-Propanol	Isooctane	Dichloromethane
Heptane	Acetonitrile	Dichloroethane
Ethyl Acetate	2-MeTHF	Chloroform
Isopropyl acetate	THF	NMP
Methanol	Xylenes	DMF
MEK	DMSO	Pyridine
1-Butanol	Acetic Acid	DMAc
t-Butanol	Ethylene Glycol	Dioxane
		Dimethoxyethane
		Benzene
		Carbon Tetrachloride

Fig. 5 Solvent selection guide.[18]

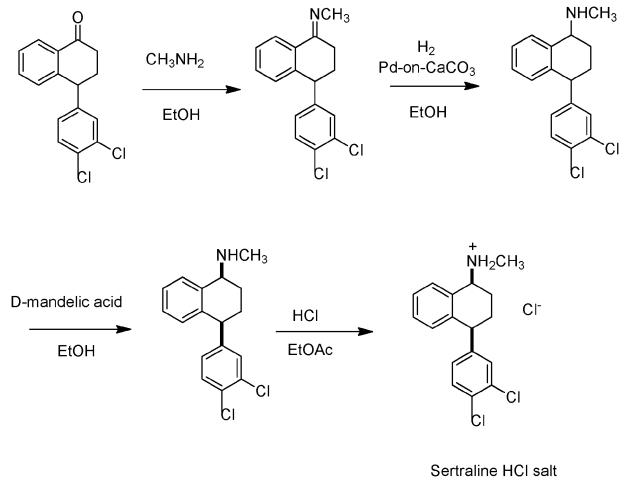

Fig. 6 Streamlined process for sertraline.

use, distil and recover four class 2 solvents: dichloromethane, tetrahydrofuran, toluene and *n*-hexane.

Since the major sources of waste in chemicals manufacture are clearly stoichiometric reagents and solvent losses the solution to the waste problem is evident: catalytic reactions in alternative reaction media.[32,33] With regard to the latter, the best solvent is no solvent but if a solvent is needed it should be safe to use and there should be provisions for its efficient separation from the product and reuse. Various non-conventional reaction media have been intensely studied in recent years, including *water*,[34] *supercritical* CO_2,[35] *fluorous biphasic*,[36] *and ionic liquids*,[37] alone or in liquid-liquid biphasic combinations.[38] The use of water and supercritical carbon dioxide as reaction media fits well with the current trend towards the use of renewable, biomass-based raw materials, which are ultimately derived from carbon dioxide and water.

7. Alcohol oxidation: a pivotal reaction in organic synthesis

The pressing need for more catalysis in organic synthesis is nowhere more apparent than in oxidative processes. For example, even in current editions of organic chemistry textbooks, the reagent of choice for the oxidation of secondary alcohols to the corresponding ketones is the Jones reagent. The latter consists of chromium trioxide in sulfuric acid, reminiscent of the phloroglucinol process referred to earlier. Nowadays, the use of stoichiometric amounts of carcinogenic chromium(VI) on an industrial scale would raise serious issues. Other stoichiometric oxidants that are popular with synthetic organic chemists are the Swern reagent[39] and the Dess-Martin periodinane.[40] The former produces the evil smelling dimethyl sulfide as the coproduct, the latter is shock sensitive, and the atom economy of both reagents is abominable (Fig. 7).

There is clearly a definite need in the fine chemical and pharmaceutical industries for (catalytic) systems that are green, scalable and have broad synthetic utility. However, as noted above, there are many shades of green. The use of NaOCl as a stoichiometric oxidant affords one equivalent of NaCl as the coproduct, and perhaps the possibility of forming chlorinated impurities, but it constitutes a dramatic improvement

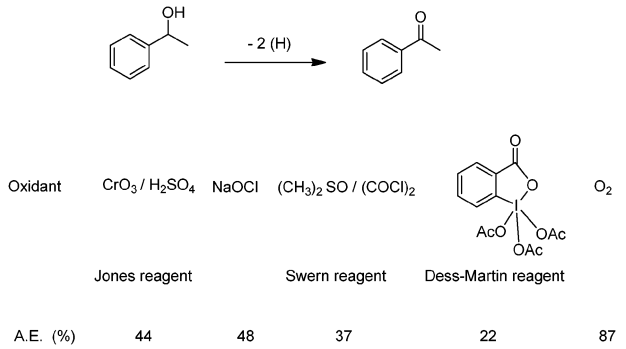

Fig. 7 Comparison of various reagents for alcohol oxidation.

compared to the chromium(VI) and other reagents referred to above. Furthermore, the scale of pharmaceuticals manufacture is such that the volumes of NaCl coproduct are not an issue. Nonetheless, catalytic methodologies employing the green oxidants, molecular oxygen (air) and hydrogen peroxide, as the terminal oxidant would seem to represent a further improvement in this respect. However, as Dunn and coworkers have pointed out,[22] the use of molecular oxygen presents significant safety issues in connection with the flammability of mixtures of oxygen with volatile organic solvents in the vapour phase. Even when these concerns are reduced by using oxygen diluted to 10% with nitrogen these methods lie on the edge of acceptability. However, an improved safety profile and more acceptable scalability can be achieved by performing the oxidation in water as an inert solvent.

7.1. Palladium catalyzed aerobic oxidation of alcohols in water

One of the reactions that Berzelius first classified as an example of what he called catalysis was the aerobic oxidation of ethanol in contact with platinum metal. As such, this was the first documented example of heterogeneous catalysis. The aerobic oxidation of alcohols and carbohydrates, over heterogeneous noble metal catalysts has in the last two centuries been extensively studied.[41] Homogeneous catalysts, on the other hand, are generally more active and selective than their heterogeneous counterparts but have the disadvantage of cumbersome recovery and reuse. This serious shortcoming of homogeneous catalysts can be overcome by performing the reaction in an aqueous biphasic system, whereby the catalyst resides in the water phase and the product is dissolved in the organic phase.[42,43] When the reaction is complete the catalyst can be recovered and recycled by simple phase separation.

Berzelius reported the stoichiometric oxidation of ethanol with K_2PdCl_4 already in 1828.[44] In the last century the use of palladium(II) salts as catalysts for the aerobic oxidation of alcohols was extensively studied.[45] However, activities were generally low, with turnover frequencies of the order of 1–10 h^{-1}. The catalytic mechanism involves the reduction of palladium to the zerovalent state by the alcohol substrate and its subsequent reoxidation to palladium(II) by dioxygen. The transient Pd(0) species is metastable and prone to aggregation to bulk palladium metal (Pd black) with concomitant loss of catalytic activity.[46] One way to avoid this is by adding coordinating ligands which stabilize the transient Pd(0) species. In 2000 we reported[47] the use of water soluble palladium complexes of chelating diamine

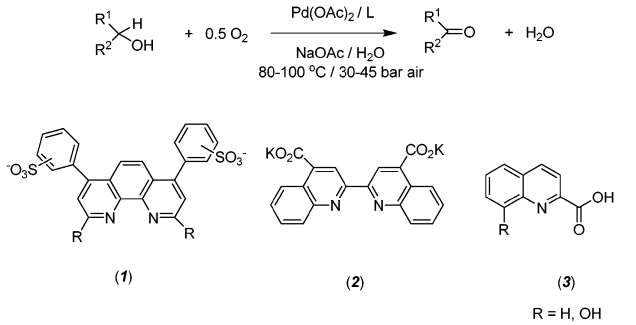

Fig. 8 Green catalytic oxidation of alcohols.

ligands based on the phenanthroline structure as active, stable and recyclable catalysts for the green aerobic oxidation of alcohols in water in the absence of organic solvents (Fig. 8).

The palladium (II) complex of bathophenanthroline sulfonate (***1***) proved to be an active catalyst (TOFs $>100h^{-1}$) for the highly selective aerobic oxidation of a variety of primary and secondary alcohols to the corresponding acids or aldehydes and ketones, respectively. Since the reaction takes place in the aqueous phase, the substrate must be at least sparingly soluble in water. A more serious drawback of the system is the low tolerance for certain (coordinating) functional groups in the substrate. Only a single ether functionality was tolerated and other functional groups containing heteroatoms, *e.g.* N or S, which coordinate more strongly to palladium, were not tolerated.

Buffin and coworkers reported[48] the use of the structurally related Pd(II) complex of the biquinoline ligand (***2***) as a catalyst for the aerobic oxidation of alcohols in water. More recently, Muldoon and coworkers[49] reported that Pd(II) complexes of chelating N,O-ligands, such as (***3***) were excellent catalysts for aerobic oxidations of neat 2-octanol. The substrate scope and, hence, functional group tolerance has yet to be examined.

A plausible catalytic cycle,[53] consistent with the observed half-order in palladium, involves initial dissociation of a hydroxyl bridged palladium(II) dimer to the monomer, which is the active catalyst. Coordination of the alcohol substrate and β-hydrogen elimination affords the carbonyl product and palladium(0) which is re-oxidized to palladium(II) by dioxygen. Further evidence in support of this mechanism has been reported by Stahl and coworkers.[50]

In a search for more active catalysts, with better functional group tolerance, we examined complexes of phenanthrolines substituted at the 2 and 9 positions.[51] We expected that this would create steric crowding in the dimer and favour its dissociation and, hence, increase its overall activity. This indeed proved to be the case and the Pd(II) complex of neocuproin (***4***) was an order of magnitude more active than the complex of (***1***) and could be used in relatively low catalyst loadings (0.1 mol%), albeit in 50/50 v/v DMSO/water. It also exhibited broad functional group tolerance, *e.g.* for O, N and S-containing moieties.

7.2. Palladium nanoparticles as catalysts for alcohol oxidation

A subsequent, more detailed examination of the results obtained with the Pd(II) bathophenthroline and Pd(II) neocuproin complexes revealed a remarkable difference in the oxidation of the

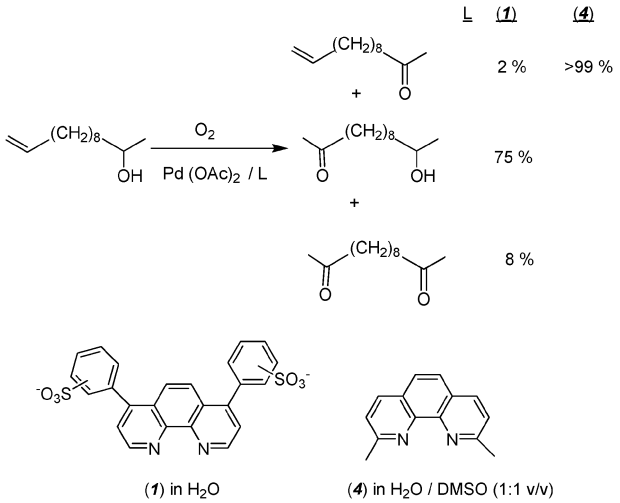

Fig. 9 Comparison of the chemoselectivities of two Pd complexes.

unsaturated alcohol substrate shown in Fig. 9. With the former the major product was derived from Wacker-type oxidation of the olefinic double bond while the latter afforded >99% selective oxidation of the alcohol moiety

The latter result bore a close resemblance to the pioneering work of Moiseev and coworkers[52] who showed that giant Pd clusters (nowadays known as Pd nanoparticles) catalyze the oxidation of alcohol moieties and selectively oxidize allylic C–H bonds in olefins. Indeed, further investigation revealed that the Pd(II) neocuproin complex dissociates completely to afford Pd nanoparticles which are the actual catalyst.[53] Indeed, the use of palladium[54] and gold[55] nanoparticles as highly active and selective catalysts for the aerobic oxidation of alcohols is now well established.

7.3. Ferritin as a nanoreactor for palladium nanoparticles

The above results led us to the idea of using the iron transport protein, ferritin, as a nanoreactor for the production and stabilization of palladium nanoparticles. Ferritins are a family of proteins,found in all organisms, the function of which is primarily to store and sequester iron. They are composed of 24 subunits that self-assemble to form a cage of 12 nm diameter in which hydrated ferric oxide (or phosphate) is formed. The apoferritin cage, formed by removing the iron (III), can accommodate cores of metallic nanoparticles such as Pd.[56] We reasoned that the introduction of palladium nanoparticles into the apoferritin cage would produce a 'semi-synthetic oxidase' for aerobic alcohol oxidation. To this end we generated palladium nanoparticles by reduction of $PdCl_2$ that had been introduced into the cage of a novel thermostable apoferritin derived from *Pyrococcus furiosus*. It proved to be a stable and recyclable catalyst for the oxidation of alcohols at 80 °C in water (Fig. 10).[57]

The Pd-ferritin catalyzed oxidation of alcohols is a good example of what we call *chemomimetic biocatalysis,* that is the design of superior catalysts by using proteins as scaffolds and building on a knowledge of chemocatalysis.

7.4. Organocatalytic oxidations with stable nitroxyl radicals

Organocatalysis is currently the focus of much attention.[58] The stable free radical, TEMPO (2,2,6,6-tetramethylpiperidinyloxyl)

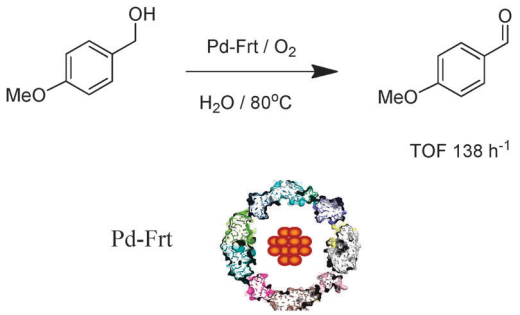

Fig. 10 Oxidation with Pd nanoparticles in a ferritin cage.

is an example of an organocatalyst that is effective in the oxidation of a broad range of alcohols,[59] including simple carbohydrates and polysaccharides,[60] using hypochlorite (household bleach) as the terminal oxidant (Fig. 11). The standard protocol, first described in 1987 by Montanari and coworkers,[61] using 1 mol% TEMPO or a derivative thereof as the catalyst, in combination with 10 mol% sodium bromide as cocatalyst, in dichloromethane/water at pH 9 and 0 °C, is now a widely used method in the fine chemicals industry.[62] The active oxidant is the oxoammonium cation which is reduced to the corresponding hydroxylamine. Oxidation of the latter by hypochlorite completes the catalytic cycle.

The Montanari protocol, although widely applicable, suffers from several environmental and/or economic drawbacks. It is not waste-free because at least one equivalent of sodium chloride is produced per molecule of alcohol oxidized and the use of hypochlorite as oxidant can also lead to the formation of chlorinated by-products. Other shortcomings are the use of 10 mol% bromide as a cocatalyst and dichloromethane as a solvent. With regard to the latter issue, we recently conducted a solvent screening study aimed at identifying greener alternatives for the N-oxy radical catalyzed oxidation of alcohols with hypochlorite.[63] We found that environmentally acceptable ester solvents, notably methyl acetate and isopropyl acetate, gave results comparable to or better than dichloromethane. A comparison of two different cocatalysts, NaBr and borax,[64] revealed that the latter gave better results with cinnamyl

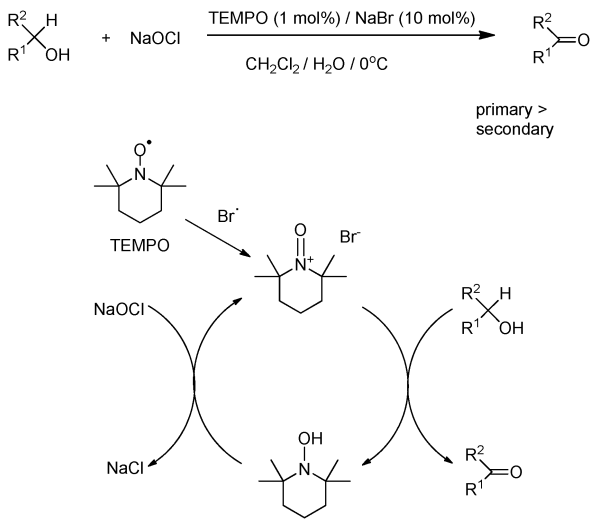

Fig. 11 TEMPO catalysed oxidations with NaOCl.

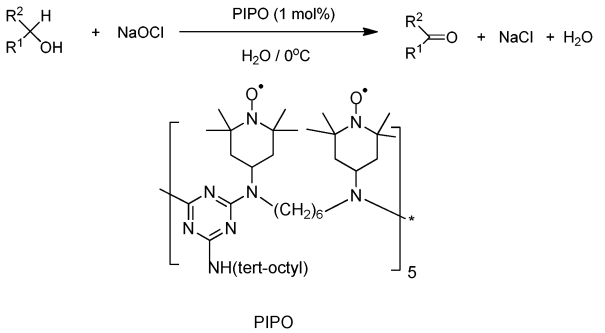

Fig. 12 PIPO-catalysed alcohol oxidation with NaOCl.

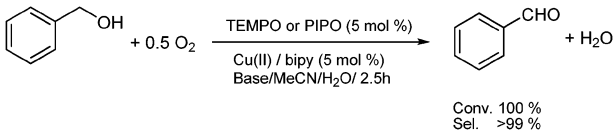

Fig. 13 Cu (bipy)/ TEMPO catalysed alcohol oxidation.

alcohols but NaBr was better with most other alcohols. In the oxidation of 3-phenyl-1-propanol the amount of N-oxy radical catalyst could be reduced to 0.1 mol%.

With a view to improving the environmental and economic features of the process, we introduced[65] the use of a recyclable oligomeric piperidinyloxyl radical, PIPO, derived from a commercially available and relatively inexpensive polymer additive, chimassorb 944. PIPO is more reactive than TEMPO, which also enables the use of more acceptable solvents, such as ethyl acetate or methyl tert-butyl ether, rather than the standard dichloromethane, and without the necessity for a bromide cocatalyst (Fig. 12).

The use of hypochlorite as the stoichiometric oxidant still remains a shortcoming in the context of waste minimization. We subsequently showed[66] that a system comprising a mixture of an N-oxy radical (TEMPO or PIPO) and a bipyridyl-Cu(II) complex, together with a base, formed an excellent catalyst for the selective aerobic oxidation of alcohols in an aqueous medium (Fig. 13).

The system displays almost complete specificity for primary *versus* secondary alcohol functionalities. Results of kinetic isotope studies were consistent with a mechanism analogous to that observed with the copper-dependent enzyme, galactose oxidase and involving an N-oxy radical coordinated to a copper(II) centre, rather than the oxoammonium cation, as the active oxidant.[67]

7.5. Chemoenzymatic oxidation of alcohols with laccase/ TEMPO

Galli and coworkers[68] showed that another copper-dependent oxidase, laccase (E,C. 1.10.3.2) in combination with TEMPO as a cocatalyst catalyzed the aerobic oxidation of primary benzylic alcohols. Laccases are extracellular enzymes that are secreted by white rot fungi and play an important role in the delignification of lignocellulose, the major constituent of wood, by these microorganisms. We have shown, by measuring isotope effects, that the reaction involves an oxoammonium cation as the active oxidant (Fig. 14), analogous to the TEMPO

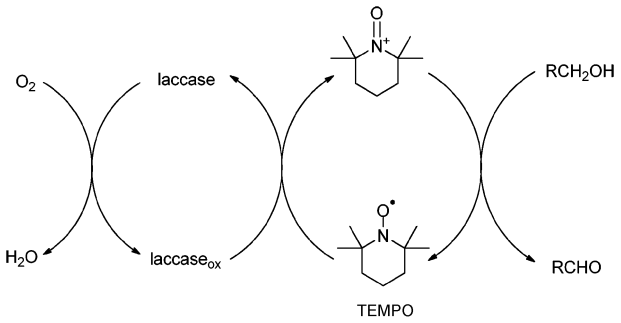

Fig. 14 Laccase/TEMPO catalysed aerobic alcohol oxidation.

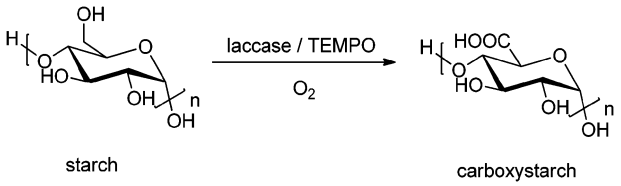

Fig. 15 Laccase/TEMPO catalysed oxidation of starch.

catalysed oxidations with NaOCl but different to the above mentioned copper/TEMPO systems.[69] The different mechanisms can be ascribed to the high redox potential of Cu(II) in fungal laccases which is needed for their *in vivo* catalysis of lignin degradation.

The laccase/TEMPO system catalyzes the selective aerobic oxidation of the primary alcohol groups in starch to afford carboxystarch (Fig. 15). A potential industrial application of the latter is as a biodegradable water super adsorbent to substitute the currently used polyacrylates.

This example serves to introduce another objective of Green Chemistry: the development of greener products. The latter should preferably be non-toxic, biodegradable and produced by a green catalytic process from renewable raw materials. Moreover, it should not cost more than the product it is replacing. In the above example, unfortunately, the relatively high enzyme costs form an obstacle to commercialization. Inefficient laccase use is a consequence of its instability towards the oxidizing reaction conditions that, in turn, is probably caused by oxidation of reactive NH_2 moieties on the exterior surface of the enzyme. Hence, we reasoned that its operational stability could be improved by cross-linking of these groups. Indeed, we found that the stability of the laccase under operating conditions is significantly improved by immobilization as a cross-linked enzyme aggregate (CLEA) (see later).

8. Carbonylation: Efficiency in C–C bond formation

As illustrated in Fig. 4, carbonylation reactions, of *e.g.* alcohols, are 100% atom efficient and, hence, constitute an attractive approach to forming C–C bonds. An elegant example of this is provided by the manufacture of the over-the-counter, non-steroid anti-inflammatory drug, ibuprofen. Two routes for the production of ibuprofen, *via* the common intermediate, p-isobutylacetophenone, are compared in Fig. 16. The classical route, developed by the Boots Pure Drug Company (the discoverers of ibuprofen), entails 6 steps with stoichiometric

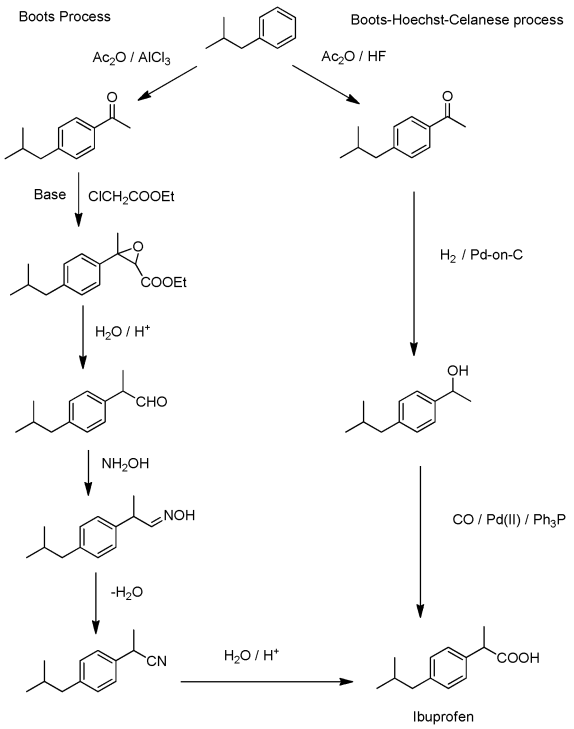

Fig. 16 Two processes for the manufacture of ibuprofen.

reagents, relatively low atom efficiency and substantial inorganic salt formation. In contrast, the elegant alternative, developed by the Boots Hoechst-Celanese (BHC) company, involves only three catalytic steps.[70] The first step involves the use of anhydrous hydrogen fluoride as both a catalyst and solvent in a Friedel-Crafts acylation. The hydrogen fluoride is recycled with >99.9% efficiency and waste is essentially eliminated. This is followed by two catalytic steps (hydrogenation and carbonylation) both of which are 100% atom efficient.

The BHC ibuprofen process was commercialized in 1992 and received a Presidential Green Chemistry Challenge Award in 1996. It represents a benchmark in environmental excellence in chemical processing technology that revolutionized bulk pharmaceutical production and became a source of inspiration for other pharmaceutical manufacturers.

The key carbonylation step involves a homogeneous palladium catalyst and contamination of the product, the active pharmaceutical ingredient, with unacceptably high amounts of palladium necessitates an expensive purification. This is a shortcoming of this almost perfect process and of homogeneous catalysis in general. Replacing the organic soluble palladium(0) triphenylphosphinecomplex with an analogous complex of the water soluble trisulfonated triphenylphosphine, tppts , affords a catalytic system for the aqueous biphasic carbonylation of alcohols.[71] Thus, when the above mentioned ibuprofen synthesis was performed with tppts in an aqueous biphasic system product contamination by the catalyst was essentially eliminated.

9. Biocatalysis: Naturally efficient

Biocatalysis has many benefits to offer in the context of green chemistry. Reactions are performed under mild conditions (physiological pH and ambient temperature and pressure)

with a biodegradable catalyst (an enzyme) that is derived from renewable resources and in an environmentally compatible solvent (water). Furthermore, reactions of multifunctional molecules proceed with chemo-, regio- and stereoselectivities and generally without the need for functional group activation, protection and deprotection steps required in traditional organic syntheses. This affords processes which are more step economic, generate less waste and are, therefore, both environmentally and economically more attractive than conventional routes. As a direct consequence of the high regio and stereoselectivities, coupled with milder reaction conditions, they often afford products in higher quality than traditional chemical or chemo-catalytic processes. For example, they avoid the problem of contamination with traces of (noble) metals which is often a serious issue in pharmaceuticals manufacture. Finally, enzymatic processes (but not fermentations) can be conducted in standard multi-purpose batch reactors and, hence, do not require any extra investment.

Advances in biotechnology over the last two decades have provided the basis for the widespread application of biocatalysis in industrial organic synthesis. Protein engineering techniques, such as *in vitro* evolution,[72] have enabled the development of tailor made enzymes exhibiting predefined substrate specificity, activity, stability, pH profile, *etc*. This enables the optimization of an enzyme to fit the pre-defined optimum process, *i.e*., truly benign by design. Furthermore, the development of effective immobilisation techniques (see later) has paved the way for optimising the storage and operational stability and the recovery and recycling of enzymes. Moreover, since most biocatalytic processes are performed under roughly the same conditions of (ambient) temperature and pressure, it is eminently feasible to integrate multiple steps into enzymatic cascade processes.[73] Co-immobilization of two or more enzymes then affords multifunctional solid biocatalysts capable of catalyzing such cascade processes.[74]

9.1. Whole cells or isolated enzymes?

Biocatalytic processes can be performed as whole cell biotransformations or with isolated enzymes. The latter have the advantage of not being contaminated with other enzymes present in the cell but the use of whole cells is less expensive as it avoids separation and purification of the enzyme. In the case of dead cells, the E Factors of the two methods are essentially the same; the waste cell debris is separated before or after the biotransformation. In contrast, when growing microbial cells are used *i.e*. in fermentation processes, substantial amounts of biomass can be generated. We note, however, that the waste biomass is generally easy to dispose of, *e.g*. as animal feed or can, in principle, be used as a source of energy for the process. Many fermentation processes also involve the formation of copious amounts of inorganic salts that may even be the major contributor to waste. However, to our knowledge there are no reported E Factors for fermentation processes. This would seem to be a hiatus which needs to be filled.

Mass balances of a few fermentation processes have been documented by Petrides[75] from which E factors can be calculated. For example the E factor for the bulk fermentation product, citric acid, is 1.4 which falls within the E factor range of <1–5 typical of bulk petrochemicals (see Table 2). Roughly 75% of the waste is accounted for by calcium sulfate. During the process calcium hydroxide is added to control the pH, affording calcium citrate which is reacted with sulfuric acid to produce citric acid and calcium sulfate. Inclusion of water in the calculation afforded an E factor of 17.

Table 2 E factors of fermentations

Product	E Factor	E factor (incl. water)
Citric acid	1.4	17
Bioethanol	1.1	42[a]
Rec. insulin	6600	50 000

[a] Includes water and carbon dioxide.

According to a recent report[76], the E factor of cellulosic ethanol is 1.1. However, if water (36.8 kg/kg ethanol) and carbon dioxide (4.1 kg/kg ethanol) are included it becomes 42. It was further noted that a cellulosic ethanol plant processing 10 000 tons of lignocellulose feedstock per day to produce 870 tons of ethanol a day would generate 32 million liters of wastewater daily which would be enough to supply a town of 300 000 inhabitants. This water contains several organic by-products, the concentrations of which have to be decreased to the ppm level or below in order to enable reuse of the water.

The fermentative manufacture of biopharmaceuticals can have very high E factors, even compared with those observed in the production of small molecule drugs. The production of recombinant human insulin,[85] for example, involves an E factor of *ca*. 6600. The most important contributors to the waste are (in kgs per kg insulin) urea (1692), acetic acid (1346), formic acid (968), phosphoric acid (713), guanidine hydrochloride (445), glucose (432), sodium chloride(430), acetonitrile (424) and sodium hydroxide (140). If water is included the E factor becomes a staggering 50 000.

In stark contrast, biotransformations involving the use of isolated enzymes generally proceed at significantly higher substrate concentrations and combine a higher productivity with a lower water usage compared to fermentations.

9.2. A green by design biotransformation

An illustrative example is the green-by-design, two-step, three-enzyme process[77,78] for the synthesis of a key intermediate (Fig. 17) in the manufacture of atorvastatin, the active ingredient of the cholesterol lowering drug Lipitor®. The process has been successfully commercialized on a multi-ton scale by Codexis. The first step involves the biocatalytic reduction of ethyl-4-chloroacetoacetate using a ketoreductase (KRED) in combination with glucose and a NADP-dependent glucose dehydrogenase (GDH) for cofactor regeneration. The (*S*) ethyl-4-chloro-3-hydroxybutyrate product was obtained in 96% isolated yield and $>99.5\%$ e.e. In the second step a halohydrin dehalogenase (HHDH) was employed to catalyze a nucleophilic substitution of chloride by cyanide using HCN at neutral pH and ambient temperature.

All previous manufacturing routes to the hydroxynitrile product (HN) involved, as the final step, a standard S_N2 substitution of halide with cyanide ion in alkaline solution at elevated temperatures. However, both substrate and product

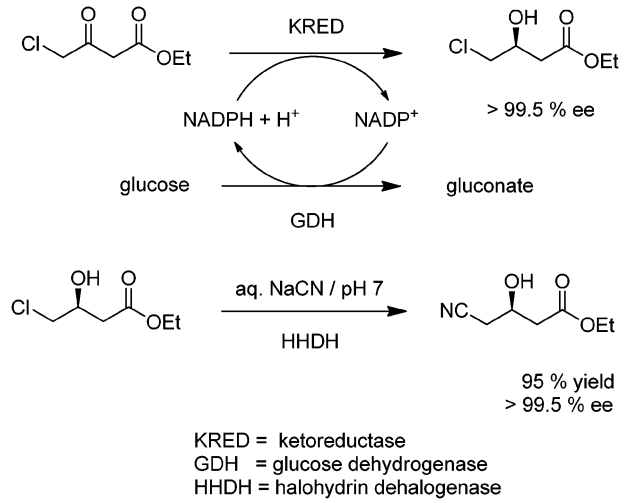

Fig. 17 A three-enzyme, two step process for atorvastatin intermediate.

are base-sensitive and extensive by-product formation is observed. To make things worse, the product is a high-boiling oil and a troublesome high-vacuum fractional distillation is required to recover product of acceptable quality, resulting in further yield losses and waste.

We reasoned that the key to designing an economically and environmentally attractive process for HN was to develop a methodology for conducting the cyanation reaction under mild conditions at neutral pH, by employing an enzyme, halohydrin dehalogenase (HHDH). Combination of this with the synthesis of the chlorohydrin substrate by highly enantioselective KRED catalyzed reduction of the corresponding keto ester, and cofactor regeneration with glucose/GDH, afforded an elegant two-step, three enzyme process. Unfortunately, the wild-type KRED and GDH exhibited prohibitively low activities and large enzyme loadings were required to obtain an economically viable reaction rate. This led to troublesome emulsion formation in downstream processing. Thus, although the analytical yield was >99% the recovered yield was only 85%. To enable a practical large-scale process the enzyme loadings needed to be drastically reduced and this was achieved with *in vitro* evolution using the DNA shuffling technique[79] to improve the activity and stability of KRED and GDH while maintaining the nearly perfect enantioselectivity exhibited by the wild-type KRED. With the improved enzymes the reaction was complete in 8 h with an increased substrate loading to 160 g L^{-1} and substantially reduced enzyme loadings and, consequently, no emulsion problems. Phase separation required less than one minute and provided the chlorohydrin in >95% isolated yield of >99.9% e.e.

Similarly, the activity of the wild-type HHDH in the non-natural cyanation reaction was extremely low and the enzyme exhibited severe product inhibition and poor stability under operating conditions. As a result of the large enzyme loadings, downstream processing was challenging. However, after many iterative rounds of DNA shuffling, the inhibition was largely overcome and the HHDH activity was increased more than 2500-fold compared to the wild-type enzyme.

The greenness of the process was assessed according to the twelve principles of green chemistry.

Principle 1 - waste prevention: The highly selective biocatalytic reactions afforded a substantial reduction in waste. In the final process, raw material is converted in >90% isolated yield to a product that is more than 98% chemically pure with an enantiomeric excess of >99.9%. Furthermore, the avoidance of by-products obviates the need for further yield-sacrificing fractional distillation. The butyl acetate and ethyl acetate solvents, used in extraction of the product from the aqueous layer in the first and second step, respectively, are recycled with an efficiency of 85%. The E Factor (kgs waste per kg product) for the overall process is 5.8 if process water is excluded (2.3 for the reduction and 3.5 for the cyanation). If process water is included the E factor for the whole process is 18 (6.6 for the reduction and 11.4 for the cyanation). The main contributors to the E factor, as shown in Table 3, are solvent (EtOAc and BuOAc) losses (51%), sodium gluconate (25%), NaCl and Na_2SO_4 (combined *ca.* 22%).

The three enzymes and the NADP account for <1% of the waste. The main waste streams are aqueous and biodegradable.

Principle 2 - atom economy: The use of glucose as the reductant for cofactor regeneration is cost effective but the atom economy is only 45%. However, glucose is a renewable resource and the gluconate co-product is fully biodegradable.

Principle 3 - less hazardous chemical syntheses: The reduction step uses starting materials that pose no toxicity to human health or the environment. It avoids the use of potentially hazardous hydrogen and heavy metal catalysts throughout the process thus obviating concern for their removal from waste streams and/or contamination of the product. While cyanide must be used in the second step, as in all practical routes to HN, it is used more efficiently (higher yield) and under less harsh conditions compared to previous processes.

Principle 4 - design safer chemicals: This principle is not applicable as the hydroxynitrile product is the commercial starting material for atorvastatin.

Principle 5 - safer solvents and auxiliaries: Safe and environmentally acceptable butyl acetate is used, together with water, as the solvent in the biocatalytic reduction reaction and extraction of the hydroxynitrile product; no auxiliaries are used.

Principle 6 and 9 - design for energy efficiency, and catalysis: In contrast with previous processes which employ elevated temperatures for the cyanation step and high pressure hydrogenation for the reduction step, both steps in the process in Fig. 17 are very efficient biocatalytic transformations. The reactions are run at or close to ambient temperature and pressure and

Table 3 E factor of the process for atorvastatin intermediate

Waste	Quantity (kg per kg HN)	% of E (excl. H_2O)	% of E (incl. H_2O)
Triethanolamine	0.04	<1%	<1%
NaCl and Na_2SO_4	1.29	22%	*ca.* 7%
Na-Gluconate	1.43	*ca.* 25%	*ca.* 9%
BuOAc (85%recycle)	0.46	*ca.* 8%	*ca.* 3%
EtOAc (85%recycle)	2.50	*ca.* 43%	*ca.* 14%
Enzymes	0.023	<1%	<1%
NADP	0.005	0.1%	<0.1%
Water	12.250	—	67%
E Factor	**5.8 (18)**[a]		

[a] Figure in parentheses includes water.

pH 7 and the very high energy demands of high vacuum distillation are dispensed with altogether, resulting in substantial energy savings. The turnover numbers for the different enzymes are $>10^5$ for KRED and GDH and $>5 \times 10^4$ for HHDH.

Principles 7 and 10 - use of renewable feedstocks, and design for degradation: The enzyme catalysts and the glucose co-substrate are derived from renewable raw materials and are completely biodegradable. The by-products of the reaction are gluconate, NADP (the cofactor that shuttles reducing equivalents from GDH to KRED) and residual glucose, enzyme, and minerals and the waste water is directly suitable for biotreatment.

Principle 8 - reduce derivatization: The process avoids derivatization steps, *i.e.* it is step economic and involves fewer unit operations than earlier processes, most notably by obviating the trouble-prone product distillation or the bisulfite mediated separation of dehydrated by-products.

Principle 11 and 12 - real time analysis for pollution prevention, and inherently safer chemistry: The reactions are run in pH-stat mode at neutral pH by computer-controlled addition of base. Gluconic acid generated in the first reaction is neutralized with aq. NaOH and HCl generated in the second step is neutralized with aq. NaCN, regenerating HCN (pKa ~ 9) *in situ*. The pH and the cumulative volume of added base are recorded in real time. Feeding NaCN on demand minimizes the overall concentration of HCN affording an inherently safer process.

In short, this process provides an excellent example of a *benign by design* biocatalytic process for the synthesis of an important pharmaceutical intermediate, the successful commercialization of which has been enabled by the employment of modern protein engineering to optimize enzyme performance. In 2006, Codexis received a Presidential Green Chemistry Challenge Award for the development of this process.

10. Enzyme immobilization for maximum efficiency

Notwithstanding the many benefits of enzyme catalysis, their commercial application is often impeded by low operational stability and shelf-life in addition to their cumbersome recovery and re-use and the product contamination that is a characteristic feature of most homogeneous catalysts. Although these problems can be alleviated by *in vitro* evolution, another approach to rendering enzymes more robust and recyclable is to immobilise them.[80] Among the several methodologies for enzyme immobilisation one that is particularly effective is immobilisation as cross-linked enzyme aggregates (CLEAs®).[81] The technique is exquisitely simple, involving standard precipitation of the enzyme from aqueous buffer, *e.g.* with ammonium sulfate, and cross linking of the resulting physical aggregates of enzyme molecules with a bifunctional reagent such as glutaraldehyde. Since selective precipitation is often used to purify enzymes 'cleation' essentially involves a combination of purification and immobilisation into a single unit operation and there is no need for the enzyme to be of high purity. Indeed, it could even be possible to isolate an enzyme in immobilised form directly from a fermentation broth.

As we have noted elsewhere,[9] brevity is the soul of synthesis. The ultimate in green catalytic methodologies is to integrate several catalytic steps into step economic, one-pot procedures

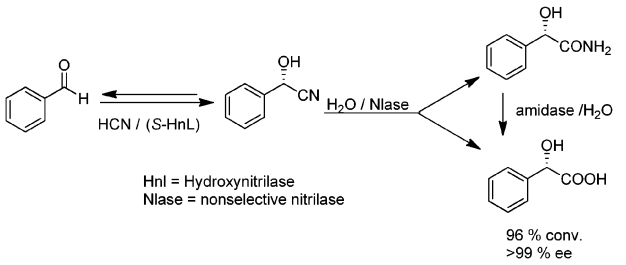

Fig. 18 A trienzymatic cascade process with a tripledecker CLEA.

without the need for isolation of intermediates.[82] This is truly emulating the elegant orchestration of enzymatic steps in metabolic pathways in the living cell. Such 'telescoping' of multi-step syntheses has several advantages: fewer unit operations, less solvent, and reactor volume, shorter cycle times, higher volumetric and space time yields and less waste (lower E factor) - which translates to substantial economic and environmental benefits. Furthermore, coupling of reactions can be used to drive equilibria towards product thus avoiding the need for excess reagents. On the other hand, there are problems to be overcome: catalysts are often incompatible with each other (*e.g.* an enzyme and a metal catalyst), rates and optimum conditions can be very different and catalyst recovery and recycle complicated. Nature solves the problem of compatibility by compartmentalisation of enzymes in different parts of the cell. Hence, compartmentalisation *via* immobilisation could be the solution to these problems in cascade processes. In this context we note that biocatalytic processes generally proceed under roughly the same conditions - in water at around ambient temperature and pressure - which facilitates their integration in cascade processes.

A pertinent example (Fig. 18) involves a trienzymatic cascade process using a triple–decker combi-CLEA containing an oxynitrilase, a nitrilase and an amidase.[83]

11. The ultimate efficiency: Renewable raw materials

The ultimate in efficient organic synthesis is surely to harness the energy of the sun to synthesize fuels and chemicals from carbon dioxide and water in a green and sustainable manner. This is what nature already does, of course, but it takes too long and fossil resources laid down as biomass millions of years ago are being consumed at a much faster rate than they can be renewed. These valuable resources are finite and will, sooner or later, run out. Indeed, the transition from an economy that is largely based on non-renewable fossil fuels as raw materials to a more sustainable biobased economy that is based on renewable resources is one of the great challenges that society faces in the 21st century. Among various sustainable energy options (solar, wind, geothermal) only biomass, which encompasses agricultural food and feed crops, dedicated energy crops and trees, agriculture and forestry residues, aquatic plants, and animal and municipal wastes, is a source of carbon-based fuels and chemicals. Hence, another important goal of green chemistry and sustainability is the substitution of fossil resources - oil, coal and natural gas - by biomass as the primary feedstock. The *utilisation of biomass for sustainable fuels and chemicals* has become a top priority on the international

political agenda. The switch from non-renewable fossil fuels to renewable biomass as a feedstock for liquid fuels and commodity chemicals will afford various economic, environmental and social benefits: (i) a more stable and secure supply of feedstocks, (ii) an environmentally beneficial reduction in the carbon footprint of chemicals and liquid fuels, and (iii) a more stable and profitable agricultural economy. Interestingly, these three major drivers of the bio-based economy constitute the three pillars of sustainability: profitability, planet and people. An additional benefit will be that many existing products will be substituted by alternatives that are inherently safer and have a reduced environmental footprint, for example, biocompatible and biodegradable plastics.

First generation biofuels (bioethanol and biodiesel) and bio-based commodity chemicals such as lactic acid and 1,3-propane diol are currently being produced from maize and edible oil seeds, such as rapeseed, as feedstocks. However, the availability of the latter is limited by the amount of fertile soil and the yield per hectare and competes, directly or indirectly, with food production, which is already effecting the price of food. It is evident that this is not a sustainable long term solution and the next generation of bio-based fuels and platform chemicals will utilise lignocellulosic biomass, inedible oilseed crops and/or microalgae as feedstocks in integrated biorefineries.

It goes without saying that processes for the conversion of these feedstocks should involve optimum utilisation of raw materials and minimisation of waste, *i.e.* have low E factors, by employing green catalytic methodologies. They should also employ environmentally friendly solvents, preferably water, alone or in combination with carbon dioxide. Ionic liquids are also of interest since they can, depending on their structure, dissolve large amounts of carbohydrates, including polysaccharides.[84] If they are also derived from renewable raw materials, are biodegradable and have low ecotoxicity all the better.

How will we know if a process for the conversion of biomass to fuels and/or chemicals is sustainable or not? Since E factors and atom economy (AE) have been widely used for assessing the environmental footprint of chemical manufacturing processes they would appear to be a good starting point for evaluating processes for biomass utilisation. However, evaluation of competing processes is fraught with various complicating factors inherent to biomass utilisation, many of which are a consequence of the enormous scale that is envisaged. The as yet unresolved 'net energy debate' illustrates the need for conceptual clarity in order to reach a consensus on meaningful metrics for biofuels. Some of the many issues that need to be addressed in order to obtain meaningful green metrics for comparing different methodologies for biomass conversion are:[85]

1. Where are the boundary limits in the cradle-gate-gate-grave-cradle cycle for our calculations?
2. How to deal with the food *vs* fuel dilemma?
3. How to take land and water use into account?

Conversion of lignocellulose could involve gasification to syn gas or enzymatic hydrolysis to a mixture of lignin and polysaccharides followed by depolymerisation of the latter to fermentable monosaccharides.[86] Metabolic pathway engineering is used to optimise the production of the required product based on the amount of substrate (glucose) consumed, *i.e.* the atom efficiciency.

Alternatively, carbohydrates can be converted to chemicals by chemocatalysis, for example, hydrogenation,[87] carbonylation[88] and oxidation.[89] For example, hydroxymethyl furfural (HMF) and levulinic acid (LA) are biomass-derived platform chemicals obtained by acid catalysed dehydration of hexoses (Fig. 19). In our original studies of carbonylations in aqueous media, catalysed by the water soluble $Pd(tppts)_3$ complex, we were interested in the carbonylation of carbohydrates as renewable raw materials and we studied HMF as a model substrate[89] HMF underwent selective carbonylation to give 5-formylfuran-2-acetic acid (FFA), as the sole carbonylation product (Fig. 19). There is currently much interest in the selective oxidation of renewable carbohydrate feedstocks over gold nanoparticle catalysts following the pioneering studies of Rossi and coworkers who showed that gold can be more selective than palladium or platinum in, for example, the oxidation of glucose to gluconic acid.[90] More recently, Taarning and Christensen reported[91] that aerobic oxidation of HMF in methanol over Au/TiO_2 afforded dimethyl furan-2,5-dicarboxylate in 98% yield.

Poliakoff and coworkers[92] showed that hydrogenation of aqueous LA over a ruthenium catalyst, in supercritical carbon dioxide as reaction medium, affords γ-valerolactone (GVL) in 100% selectivity (Fig. 19). The LA partitions into the aqueous phase and the GVL into the carbon dioxide phase. Alternatively, the hydrogen could be replaced by the formic acid generated as a byproduct in the formation of LA from HMF. Horvath has proposed GVL as an ideal sustainable liquid fuel and platform chemical.[93]

The shift from oil to renewable raw materials will have far-reaching consequences for the commodity chemical industry. The structure of chemical supply chains will be radically altered, creating new opportunities for innovation in green chemistry and sustainable technologies. For example, a direct consequence of the recent enormous increase in biodiesel production is that the coproduct, glycerol, has become a low-priced commodity chemical which is an interesting raw material for other bulk chemicals such as 1,2- and 1,3-propane

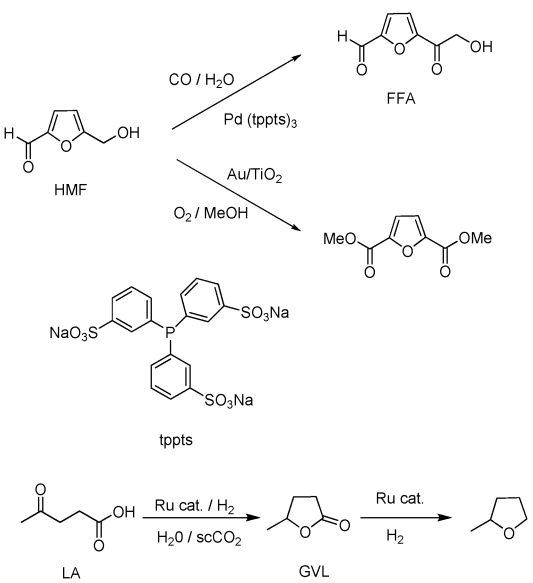

Fig. 19 Catalytic conversion of renewable raw materials.

diol and acrylic acid by catalytic reduction and oxidation, respectively. These processes will also have to be efficient in raw material utilisation and generate minimum waste.

Conclusions

Twenty years ago it was clear that a new paradigm for efficiency in organic synthesis was needed. The introduction of the principles of clean or green chemistry and the underlying concepts of waste minimization, E factors, and atom efficiency provided an answer to this need. Now, twenty years later these concepts are accepted in academic and industrial circles on a world wide basis. Substantial reductions in waste generation have been achieved by replacing outdated processes employing stoichiometric reagents with greener catalytic alternatives. The next phase of designing a more efficient and sustainable chemical industry will be the successful application of these green catalytic technologies in the efficient synthesis of organic chemicals from renewable resources.

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