

SPECTROSCOPY

Prepared by: Lee Siang Hua (HT033441Y)
Prepared for: Assoc. Prof. Marc Garland
Date of submission: 2 February 2005

*Dept. of Chemical & Biomolecular Engineering,
National University of Singapore,
10, Kent Ridge Crescent, Singapore 119260
Email: g0302120@nus.edu.sg*

BACKGROUND

In the pharmaceuticals and fine chemicals processes, yield is mainly determined by the reaction stage. A typically pharmaceutical synthesis consist about 9 reaction steps. It is impossible to have 100% conversion in any reaction steps, this result to low overall yield due to low conversion and poor selectivity. The selectivity of each reaction steps can be improved by adding catalyst and proper control of reaction conditions. By increasing the selectivity, the final yield will be improved tremendously.

The greatest challenge in chemical reaction control is to achieve the most optimum reaction conditions inclusive of proper control of catalyst concentration, reactant concentration, product concentration and by-product concentration. The reaction pressure and temperature is also very important. Typical industrial practice is to collect sample in the reactor routinely and manually analyze the concentrations of each components in the sample. This conventional method is very time-consuming, due to the time delay, process optimization is very difficult to achieve.

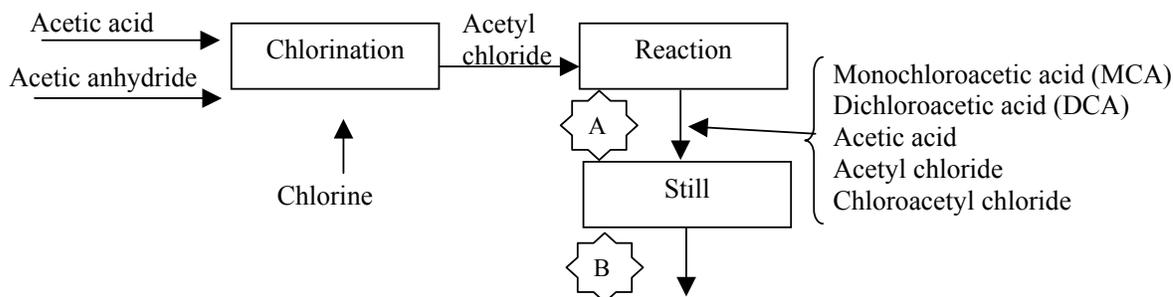
In-situ or on-line sample analyzer is preferable in the modern industry. The reaction conditions can be further optimized based on the instant result provided by on-line analyzer. However, there are some limitation in the on-line analyzing methods, such as proper sampling method, accuracy of the analyzer results and cost effectiveness of the hardware and expertise. The accuracy of the analyzer is most important because it will affect the overall control of the reactions.

The accuracy of on-line analyzer is determined by the selection of proper analysis method. A good analysis method should have high sensitivity to the species-of-interest, better time resolution, accuracy and low maintenance costs.

SUMMARY OF SELECTED PAPER

In the selected paper (Wayers et al, 2004); a 785 nm laser dispersive Raman system was installed in a monochloroacetic acid (MCA) production plant. The Raman system allowed the process to be better controlled, and making more consistent product quality. The MCA product purity was improved via a drop in the by-product dichloroacetic acid (DCA) of about 25%.

The manufacturing process was summarized as the block diagram below:



MCA is the desired product, DCA is the undesired by-product and the intermediate product acetyl chloride is the catalyst. In order to maximize the selectivity of MCA, the concentration of acetyl chloride and MCA in the reactor and the still need to be closely monitored. Table 1 is the concentrations range for each components.

Table 1. Concentration calibration range of stream A and B

Components	Stream A-reactor	Stream B-still effluent
MCA (desired product)	40-80%	95-100%
DCA (undesired by-product)	1-4.5%	0-5%
Acetic acid	15-50%	0-4%
Acetyl chloride (catalyst)	0.7-5.5%	-
Chloroacetyl chloride	0.8-3.5%	-

Before installation of the Raman system, percent conversion of MCA is routinely estimated every two hours, and catalyst content was measured only every eight hours. The limitations of the existing sampling procedure are:

- (i) Potential of operator exposed to high toxic and corrosive liquid.
- (ii) Poor estimation of MCA conversion by checking the specific gravity of the mixture of MCA, DCA and chloroacetyl chloride.
- (iii) Poor estimation of catalyst content by titration that summed the acetyl chloride and chloroacetyl chloride.
- (iv) Time delay of one shift to detect the process changes that affect the catalyst level.
- (v) Difficulty in process optimization due to time-delay and inaccuracies of available analysis.

The installation of Multiplex Raman system has overcome the above mentioned limitations:

- (i) Sampling is remote from 200 m away using four fiber-optic lines for four sampling points.
- (ii) Improved accuracy of analysis after several calibrations to the spectra.
- (iii) No time delay, thus the process can be optimized and improved the yield of MCA.

In conclusion, the multiplexed optical fiber Raman system has provide the process with reliable real-time data resulting in considerable cost savings and improved product purity, and has lowered the exposure of operators to a toxic substance.

CURRENT AND FUTURE DEVELOPMENT

In the last ten years, instrumental advances have made Raman spectroscopy an attractive technique for chemical analysis. Improved lasers, charge-coupled device (CCD) detectors, and wavelength separating filters have increased the signal-to-noise and ease of use of this technique. In addition, the ability of the technique to employ long-distance optical fibers and to view samples through glass windows has made it particularly interesting to the process analyzer community. The application of Raman spectroscopy to the monitoring of chemical processes has been reported by numerous workers in both laboratory and plant process situation. Table 2 showed the list of successful Raman Spectroscopy system used as on-line analyzer to improve the process control and optimization.

Table 2. Application of Raman Spectroscopy in on-line Process Monitoring

Year	Species-of-interest	Reported by	Other methods considered	Advantages of Raman Spectroscopy
2004	Monochloroacetic acid (liquid phase)	Weyer, et al.	MIR, autotitration, GC, NIR, NMR	Uncontactable sampling method, multiplexed sampling, sufficient discriminatory capability

2002	2-Butyl crotonate	McGill, et al.	NIR, UV/VIS, NMR	Shorter time resolution than NIR
2001	Titanium Dioxide (solid phase)	Clegg, et al.	-	Interface easily accomplished, many sample types can be analyzed, minimal intrusion to the process, spectrometer is protected, hazardous samples can be readily sampled.
1999	Para-, ortho- and meta-xylene	Gresham, et al.	NIR	Similar performance
1999	Glucose	Shaw, et al.	Couple with chemometrics	Accurate
1999	Ethyl acetate	Svensson, et al.	Couple with chemometrics	Fast and accurate
1998	chlorosilane monomers (liquid phase)	Lipp and Gross	GC	Superior time resolution

From the reported cases shown in Table 2, it is very clear that Raman Spectroscopy has a very high potential in on-line process monitoring. Even though it has higher investment cost compared to other techniques, for example the cost in parts for the NIR instrument is approximately half that of the Raman instrument (Gresham, et al: 1999), it will be the choice of instrument due to the advantages stated in Table 2.

REFERENCES

- Clegg, I.M., Everall, N.J., King, B., Melvin, H, and Norton, C. 2001. On-line Analysis Using Raman Spectroscopy for Process Control during the Manufacturing of Titanium Dioxide. *Applied Spectroscopic*. [online], 55 (9), 1138-1150. Available from: <http://thesis.ingentaselect.com.libproxyl.nus.edu.sg> [Accessed 30 Jan 2005]
- Gresham, C.A, Gilmore, D.A. and Denton, M.B. 1999. Direct Comparison of Near-infrared Absorbance Spectroscopy with Raman Scattering Spectroscopy for the Quantitative Analysis of Xylene Isomer Mixtures. *Applied Spectroscopic*. [online], 53 (10), 1177-1182. Available from: <http://thesis.ingentaselect.com.libproxyl.nus.edu.sg> [Accessed 30 Jan 2005]
- Lipp, E.D. and Gross, R.L. 1998. On-line Monitoring of Chlorosilane Streams by Raman Spectroscopy. *Applied Spectroscopic*. [online], 52 (1), 42-46. Available from: <http://thesis.ingentaselect.com.libproxyl.nus.edu.sg> [Accessed 30 Jan 2005]
- McGill, C.A., Nordon, A. and Littlejohn, D. 2002. Comparison of in-line NIR, Raman and UV-visible spectrometries, and at-line NMR spectrometry for the monitoring of an esterification reaction. *Analyst*. [online], 127, 287-292. Available from: <http://www.swetwise.com.libproxyl.nus.edu.sg> [Accessed 30 Jan 2005]
- Schmidt, K.J., Zhang, S.L., Michaelian, K.H., Webb, M.A. and Lopnow, G.R. 1999. Quantitation of Sulfur Oxyanions in Industrial Metal Refining Solutions with Dispersive and Fourier Transform Raman Spectroscopy. *Applied Spectroscopic*. [online], 53 (10), 1206-1211. Available from: <http://thesis.ingentaselect.com.libproxyl.nus.edu.sg> [Accessed 30 Jan 2005]
- Shaw, A.D., Kaderbhai, N., Jones, A., Woodward, A.M., Goodacre, R., Rowland, J.J. and Kell, D.B. 1999. Noninvasive, On-line Monitoring of the Biotransformation by Yeast of Glucose to Ethanol Using Dispersive Raman Spectroscopy and Chemometrics. *Applied Spectroscopic*. [online], 53 (11), 1419-1428. Available from: <http://thesis.ingentaselect.com.libproxyl.nus.edu.sg> [Accessed 30 Jan 2005]
- Svensson, O., Josefson, M. and Langkilde, F.W. 1999. Reaction Monitoring using Raman Spectroscopy and Chemometrics. *Chemometrics and Intelligent Laboratory Systems*. [online], 49, 49-66. Available from: <http://www.sciencedirect.com.libproxyl.nus.edu.sg> [Accessed 30 Jan 2005]
- Weyers, L.G., Yee, G and Wade, J. 2004. Installation of a Multiplexed Raman System in a Chemical Process. *Applied Spectroscopic*. [online], 58 (12), 1500-1505. Available from: <http://thesis.ingentaselect.com.libproxyl.nus.edu.sg> [Accessed 22 Jan 2005]

CATALYSIS

“ASYMMETRIC HYDROGENATION”

Prepared by: Lee Siang Hua (HT033441Y)
Prepared for: Assoc. Prof. Marc Garland
Date of submission: 2 March 2005

*Dept. of Chemical & Biomolecular Engineering,
National University of Singapore,
10, Kent Ridge Crescent, Singapore 119260
Email: g0302120@nus.edu.sg*

BACKGROUND

The mechanism of homogeneous catalysis is more accessible to detailed investigation than that of heterogeneous catalysis because the interpretation of rate data is frequently easier. Moreover, species in solution are often easier to characterize than those on a surface.

From a practical standpoint, homogeneous catalysis is attractive because it is often highly selective towards the formation of a desired product. In large-scale industrial processes homogeneous catalysts are preferred for exothermic reactions because it is easier to dissipate heat from a solution than from the solid bed of a heterogeneous catalyst.

Among the wide scope of homogeneous catalysis, asymmetric hydrogenation with homogeneous transition metal catalysts is a powerful tool for the production of optically active organic compounds. One of the most studied catalytic systems is the Rh(I) complex $[\text{RhCl}(\text{PPh}_3)_3]$ known as ‘Wilkinson’s catalyst’. The Wilkinson’s catalyst and related Rh(I) phosphine catalysts that contain a chiral phosphine ligand have been developed to synthesize optically active products in enantioselective reactions. It has proven its value in industry, e.g. the Monsanto L-DOPA process and the Takasago carbapenem process. For example, an enantioselective hydrogenation catalyst containing a chiral phosphine ligand referred to as DIPAMP is used by Monsanto to synthesize L-dopa (a chiral amino acid used to treat Parkinson’s disease). An interesting detail of the process is that the minor diastereomer in solution lead to the major product. The explanation of the greater turnover frequency of the minor isomer lies in the difference in activation Gibbs energy as shown in Figure 1. Spurred by clever ligand design, this field is growing rapidly and providing clinically useful compounds.

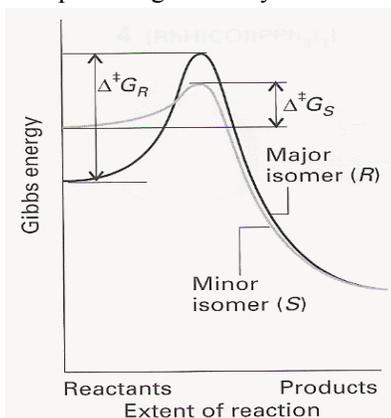


Figure 1. Kinetically controlled Stereoselectivity
(Shriver and Atkin: 1999, pp.593)

SUMMARY OF SELECTED PAPER

In this paper, the author reviews the research progress of asymmetric hydrogenation catalysis in terms of the ligands used, the reaction conditions, the substrates, and mechanisms.

Ligands

The enantiomeric excess (ee) for different rhodium-phosphine ligands has been compared for the asymmetric catalysis in the equation:



It is shown that DIPAMP (Di-*o*-methoxyphenyl) has highest ee. among the other ligands.

Table 1. Genesis of Phosphines

Ligands	Enantiomeric excess
Methylpropylphenylphosphine	28%
<i>o</i> -methoxyphenyl (PAMP)	50-60%
Cyclohexyl- <i>o</i> -anisylmethylphosphine (CAMP)	80-88%
Di- <i>o</i> -methoxyphenyl (DIPAMP)	95%

Some other phosphine with relatively high %ee (more than 80%ee) on (*Z*)- α -acetamidocinnamic are R-CAMP, R,R-DIOP, R,R-DIPAMP, Chiraphos, BPPFA, BPPM, Rhone-Poulenc and PNNP.

Reaction Conditions: Pressure, Temperature, catalyst poison, safety, chiral multiplication

Usual conditions of reaction are about 3 atm pressure and 50°C with about 1000/1 mol ratio of substrate to catalyst in aqueous ethanol or 2-propanol. Generally, higher alcohols give marginally better efficiency than methanol. In all cases the efficiency drops with pressure. This problem can be resolved using triethylamine or other base to generate anion, and the slower hydrogenation rate due to the base can be offset by running at 25-50 atm. In some instances with the anion it is only possible to get high ee's at 0°C where reaction rates are impractically slow. The hydrogenation is poisoned by oxygen or peroxides. These impurities must be removed for efficient catalyst usage. One advantage of the homogeneous asymmetric hydrogenation compared to heterogeneous is it does not catalyze the reaction of hydrogen or solvent vapors with oxygen thus not pyrophoric. This property is important in operational safety. This catalyst in the absence of oxygen or peroxide are very active, it is easily possible to make thousands of moles of product per mole of chiral agent. This enormous multiplier effect easily offset the high cost of the catalyst.

Substrates

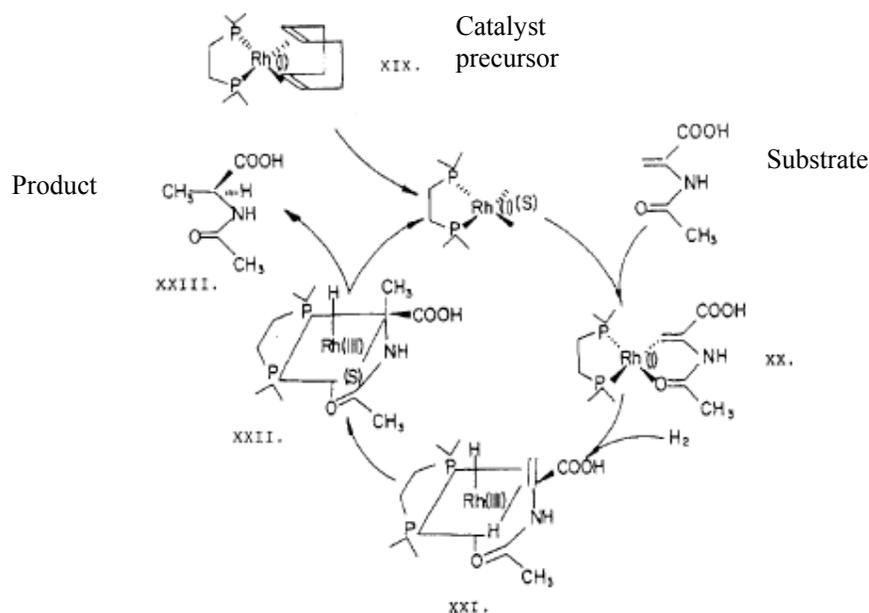
The behavior of above mentioned ligands on (*Z*)- α -acetamidocinnamic will change once varies the substituents on the olefin. Whenever an olefin substrate has the capability of forming chelate with a metal, then DIPAMP is the most generally applicable ligand. If considering simple olefins such as α -phenylacrylic acid or substituted prochiral styrenes, then DIOP is the best choice. The changes and the effect is show in Table 2.

Table 2. Ligands behave to different substrates

Changes	Effects
Carbonyl group change to ester, amide, or nitrile	DIOP inefficient
Aromatics substituent change to aliphatic	Only DIPAMP is efficient
Carbonyl group change to trifluoromethyl group	Only DIPAMP is efficient

Mechanism

The chiral phosphine and the hydrogen must all be on the metal at the same time. The catalyst must show a considerable preference for adding hydrogen to the *re* face of the olefin. Figure 2 summarized the mechanism of hydrogenation. It is observed that the stereochemical control occurs at either the hydrogen addition (XXI) or the rhodium alkyl hydride (XXII) stage. The greater ease of forming XXII with its *re* face on the metal rather than the *si* face provides a reasonable explanation of the steric control.



Satge	Mechanism
XIX	The catalyst precursor in presents of H ₂ losses cyclooctadiene, formed solvated complex containing no H ₂
XX	Substrate comes in forming square-planar rhodium(I) complex with the diastereoisomers derived from the prochiral olefin in rapid equilibrium as compared with the hydrogenation rate
XXI	H ₂ oxidatively adds to make octahedral rhodium (III) complex
XXII	The dihydride immediately converts to half-adduct stage
XXIII	Collapse to product and regeneration of solvated complex

Figure 2. Mechanism Summary

CURRENT AND FUTURE DEVELOPMENT

The commercial success of the enantioselective catalysis using metal complex is listed in Table 3.

Table 3. Commercial applications of enantioselective catalysis using metal complexes (Nugent et al: 1993)

Company	Metal	Reaction type	Ultimate product
Monsanto	Rh	Hydrogenation	L-Dopa
Sumitomo	Cu	Cyclopropanation	Cilastatin
Anic, Enichem	Rh	Hydrogenation	L-Phenylalanine
J. T. Baker	Ti	Epoxidation	Disparlure
ARCO	Ti	Epoxidation	Clycidols
Takasago	Rh	Rearrangement	L-Menthol
Merck	B	C=O reduction	MK-417 (ophthalmic)
E. Merck	Mn	Epoxidation	Antihypertensive
Takasago	Ru	Hydrogenation	Carbapenem

Among the processes, asymmetric hydrogenation is at utmost important where more than 30% of the chemical synthesis is from homogeneous asymmetric hydrogenation. One problem for broader industrial

application is the separation and recycling of catalyst, which are the complexes of costly metals. One such approach is to immobilize the catalyst.

Immobilised Catalyst (Catalyst Membrane)

Simon et al (2003) proposed a possible approach to overcome the separation and recycling problem in industry by immobilisation of the cationic catalysts on anionic supports in a membrane configuration. With the membrane there is possibility to perform two consecutive steps in one reactor. To demonstrate this principle the hydrogenation and hydrolysis steps of the L-DOPA synthesis will be performed in one pot as depicted in Figure 3.

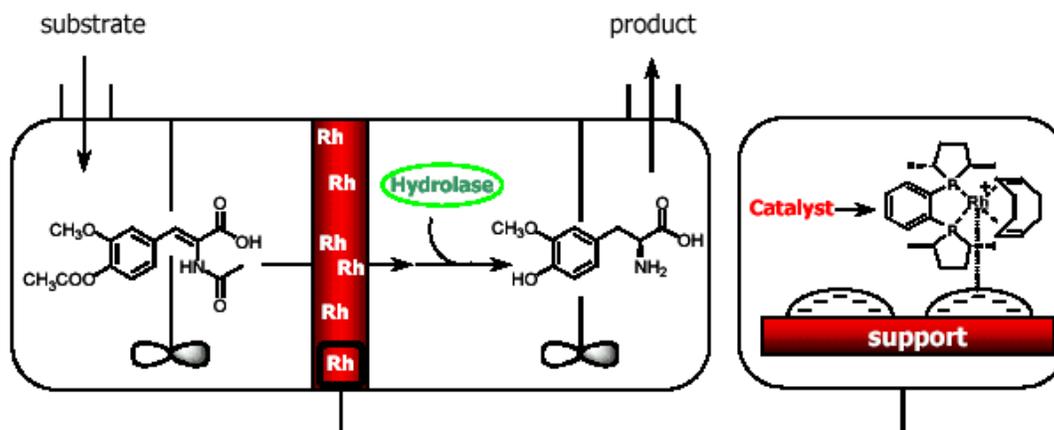


Figure 3. The asymmetric hydrogenation and hydrolysis steps of the L-DOPA synthesis carried out in a concept reactor

The immobilisation by ionic interaction has the advantage that proven homogeneous systems can directly be immobilised without difficult ligand modification. Well-known systems, like Rh-MeDuPHOS and Ru-BINAP will be immobilised on functionalised TUD-1. TUD-1 is a well-defined mesoporous (siliceous) oxide, with high surface areas, easily tuneable pore size distribution and three-dimensional connectivity. It will be functionalized with Nafion or heteropoly acids (HPA). After immobilisation of the catalyst, the materials will be tested on activity and selectivity on various substrates, mostly dehydroamino acids. Special attention is being paid to reusability and the occurrence of leaching. In the final part of the project the asymmetric hydrogenation with these catalysts will be combined with an enzyme-catalysed deacylation leading to e.g. L-DOPA.

REFERENCES

- Knowles, W.S. 1983. "Asymmetric Hydrogenation". *Acc. Chem. Res.* Vol. 16, pp. 106-112.
- Nugent, W.A, RajanBabu, T.V. and Burk, M.J. 1993. "Beyond Nature's Chiral Pool: Enantioselective Catalysis in Industry". *Science*, vol. 259, No. 5094 pp. 479-483.
- Shriver, D.F. and Atkins, P.W. 1999. *Inorganic Chemistry*. UK: Oxford University Press.
- Simon, C et al. 2003. *Catalytic membranes for asymmetric hydrogenation*. [on-line]. Available from: www.bt.tudelft.nl/content/boc/pdf/BocprSimons.pdf [Accessed 24 Feb. 2005]

UNIT OPERATION-MICRONIZATION

“Micron-Size Drug Particles: Common and Novel Micronization Techniques”

Prepared by: Lee Siang Hua (HT033441Y)
Prepared for: Assoc. Prof. Marc Garland
Date of submission: 16 March 2005

*Dept. of Chemical & Biomolecular Engineering,
National University of Singapore,
10, Kent Ridge Crescent, Singapore 119260
Email: g0302120@nus.edu.sg*

BACKGROUND

In the pharmaceutical industry, the drug particle size is an important factor affecting the drug characteristics and efficacy. This is particularly important for substances with low solubility and low absorption rate at the application site. Drugs with poor water solubility are often used in micronized particle size ($<10\mu\text{m}$) (Müller and Rasenack: 2004). In addition, there is always a predetermined particle size range suitable for pulmonary deposition of pharmaceutical aerosols, such as dry powder inhaler used for asthma treatment.

The advantages of micronized size particles are listed as follows:

1. Improvement in drug bioavailability of poorly soluble substances (solubility $< 1 \text{ mg/mL}$) due to increased dissolution rate.
2. The diffusion layer around small particles is thinner, especially when the particle size is lower than $5 \mu\text{m}$, which results in a faster distribution of the dissolved molecules.
3. The rate of absorption is not influenced by the hydrodynamic in the gastrointestinal tract, which affects the thickness of the diffusion layer.

The two major micronization technologies are mechanical size reduction after crystallization and the controlled particle size during production. The challenges for mechanical size reductions are as follows:

1. Limited control of particle size, shape, morphology, surface properties, electrostatic charge, oxidation, degradation, aggregation, cohesion and adhesion.
2. Agglomeration after micronization process.
3. Broad particle size distribution.
4. Heterogeneous particle shape.
5. Disruption of crystal lattice.
6. Disorder surface.
7. Further size reduction due to postmicronization stress relaxation.
8. Chemical degradation during milling.

Whereas, the major challenges faced for controlled particle size process are as follows:

1. Control of particle growth using stabilizing agent.
2. High production cost.
3. Environmental problem.

In the manufacturer's point of view, technology with reasonable operating cost and capital cost while producing desirable results is at utmost important. Each technology is selected based on the application and particle properties required. Some pharmaceutical manufacturer in Singapore, such as Schering Plough is using mechanical method, i.e. the fluid energy mill is used to produce their synthetic chemical and steroid Active Pharmaceutical Ingredient (API), while freeze drying techniques is used in their biological API.

SUMMARY OF SELECTED PAPER

In their paper, Müller and Rasenack (2004) had compiled the developments of micronization technology over the past thirty years (1974-2003), the advantages and challenges in each techniques was discussed, and the common and novel technique had been compared.

The micronization technologies had been divided into two major categories: breaking (comminution) of large particles using milling process such as jet milling, pearl-ball milling or high-pressure homogenization and the production of small particles using controlled production processes such as spray drying, precipitation from supercritical fluid (SCF) and controlled crystallization. These two categories are well illustrated in Figure 1.

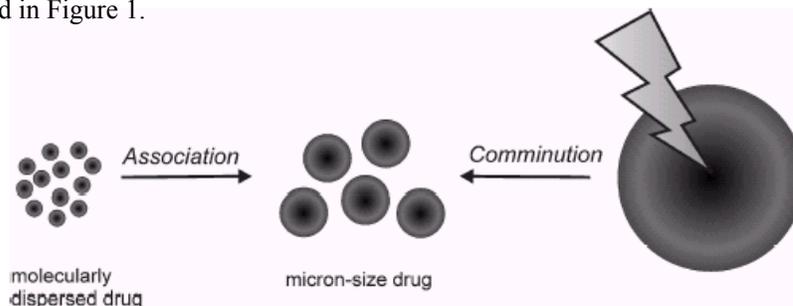


Figure 1. Illustration of the two principle methods for the production of a micron-size drug powder.

Each method had been discussed in the basic operating principle, the result, the advantages and disadvantages. Table 1 summarized the discussions of all major methods available in industry..

Table 1. Summary of Micronization Technology

Mechanical Comminution		
Jet mills/Fluid-energy mill	Process	Conversion of high pressure into kinetic energy. Milling pressure between 3-10 bar. Four basic designs: spiral, opposed, loop, target. Three collision geometrics: inter particle collisions due to turbulence in a free jet, collisions between particles accelerated by opposed jet, impact of particles on a target.
	Performance	Average particle size~1.5-5 μm , 90%<10 μm . Also available for 99% <5 μm .
High-Pressure Homogenizer	Process	A suspension is homogenized at pressure ~ 1000 bar with up to 10 cycles, causing a high strain of the drug. Due to the rapid expansion, a disruption occurs by cavitation.
	Performance	Particle size 200-400nm. Mostly amorphous particles.
Pearl-ball mill	Process	A rotating vessel, 74.4% filled with pearl-ball, moved using a rotator. Comminution due to attrition and impact.
	Performance	Crystalline products.
Challenges of these method	<ol style="list-style-type: none"> 1. Crystal cleaves at the crystal face with smallest attachment energy, thus the surface properties is dominated by this crystal face, which might be hydrophobic. To increase the dissolution rate, surfactant is needed. 2. Particles are electronically charged and further agglomeration due to their cohesive behavior. 3. Broad particle size distributions and heterogeneous shape. 4. High energy input caused disruption of crystal lattice. 5. Further size reduction due to postmicronization stress relaxation. 6. Chemical degradation, change in blending properties and dissolution properties, etc. 	

Particle Technology		
Spray Drying	Process	Spray-drying a drug solution to achieve finely dispersed drug particles.
	Performance	Small and homogeneous particle size.
	Challenges	Good for water-soluble drugs. For poor water solubility drug, organic solvent will increased operating cost and caused environmental problems.
SCF (Super Critical Fluid Technology)	Process	RESS (rapid expansion of supercritical solutions technique) : For drug soluble in supercritical CO ₂ , drug particles can be precipitated by rapid expansion of the SCF. ASES (aerosol solvent extraction system process): For limited solubility in supercritical CO ₂ . The drug is dissolved in a solvent and precipitate in the SCF.
	Performance	Uniform particle size and shape.
	Challenges	High operating cost.
Controlled Crystallization-solvent change process	Process	Precipitating the drug by a liquid solvent change process (salting out). Stabilizing agent required (e.g. poloxamer and gelatin). Spray-drying or freeze-drying to obtain stable product.
	Performance	Mean particle size 270 nm.
	Challenges	High content of stabilizing agent caused low dissolution rate.
In-situ Micronization	Process	Precipitation from drug solution by solvent change process in the presence of protective hydrophilic polymer followed by spray-drying.
	Performance	Applicable for both water-soluble and water-insoluble drugs. Homogeneous particle size distribution.
	Challenges	N/A
EPAS (evaporative precipitation into aqueous solution)	Process	Drug solution dissolved in dichloromethane or ether is heated above boiling point under pressure. The solution is sprayed into a heated aqueous phase. The resulting aqueous suspension is then spray-dried or freeze-dried.
	Performance	Increased wettability, small particle size and reduced crystallinity.
	Challenges	N/A
Spray freezing into liquid	Process	A drug solution is sprayed into liquid nitrogen, after freeze-drying the solidified droplets, the drug is obtained in micron-size form.
	Performance	Suitable for production of peptide particles.
	Challenges	N/A

CURRENT AND FUTURE DEVELOPMENT

Manufacturing of drug products is a challenge besides Research and development in this field. The economic and efficiency of each processing steps need to be balanced up with the stringent specification of the drug products. Controlling the drug particle size is at utmost important because it will affect the drug solubility and thus the effectiveness to human body.

The methods listed in Table 1 are used in the current manufacturing processes and further improvement is on-going, especially in the particle engineering field. One of the recent studies reported by Berggren, et. al (2004) shown that the compression behaviour and tablet-forming ability of spray-dried amorphous lactose can be modulated by the addition of stabilizing polymers or surfactants to the spray feed solution. Another studies in 2004 (Bandi, et.al.) proposed a solvent-free single-step SCF approach that provides

high final product yields ($\geq 96\%$) for the preparation of budesonide– and indomethacin–HPBCD complexes. Zijlstra, et. al. (2004) showed that it is possible for a stable peptide to be micronized with milling, spray drying and spray freeze drying without degradation of the drug. Whether a micronization technique produces particles that are suitable for use in an inhalation formulation was found to depend on the type of formulation and formulation process used. Furthermore, the performance of the different formulations (aerosolization behaviour expressed as fine particle fraction) was found to depend on the efficiency of the de-agglomeration principle applied. Such an inhalation system could be further developed to be a non-invasive alternative for routine administration of cetorelix.

Different drugs had been tested using different micronization methods; with different stabilization agent and solvent. The ultimate target is to obtain the optimum operating conditions with reasonable economic prospect for the micronization technologies.

REFERENCES

- Bandi, N., William, W., Christopher, B. R., Kotra, L.P., and Kompella, U.B. 2004. “Preparation of budesonide– and indomethacin–hydroxypropyl- β -cyclodextrin (HPBCD) complexes using a single-step, organic-solvent-free supercritical fluid process”. *European Journal of Pharmaceutical Sciences*. vol. 23 pp.159–168.
- Berggren, J, Frenning, G. and Alderborn, G. 2004. “Compression behaviour and tablet-forming ability of spray-dried amorphous composite particles” *European Journal of Pharmaceutical Sciences* vol. 22 , pp.191–200
- JetPharma SA Jetmill product brochure.
- Krummer, K. and Ong, S.L. 2001. “Micronizer” Thesis for the Joint Masters Program between NUS and UIUC, Unpublished.
- Lizio, R., et al. 2001. “Low Temperature Micronization of a Peptide Drug in Fluid Propellant: Case Study Cetorelix”. *AAPS PharmaSciTech*. 2(3) article 12. [On-line]. Available from: <http://www.aapspharmsciotech.org/articles/pt0203/pt020312/pt020312.pdf> [Accessed 12 March 2005].
- Müller, B.W. and Rasenack, N. 2004. “Micron-Size Drug Particles: Common and Novel Micronization Techniques”. *Pharmaceutical Development and Technology*. Vol. 9, No. 1, pp. 1-13.
- Zijlstra, G.S., Hinrichs, W.L.J, A.H. de Boer, and Frijlink, H.W. 2004. “The role of particle engineering in relation to formulation and de-agglomeration principle in the development of a dry powder formulation for inhalation of cetorelix”. *European Journal of Pharmaceutical Sciences*. Vol. 23, pp.139–149.

ENVIRONMENT ASPECTS

“PHOTOCATALYTIC DEGRADATION FOR ENVIRONMENTAL APPLICATIONS – A REVIEW”

Prepared by: Lee Siang Hua (HT033441Y)

Prepared for: Assoc. Prof. Marc Garland

Date of submission: 30 March 2005

*Dept. of Chemical & Biomolecular Engineering,
National University of Singapore,
10, Kent Ridge Crescent, Singapore 119260
Email: g0302120@nus.edu.sg*

BACKGROUND

All of the pharmaceutical compounds are complicated in structures. The difficulties of synthesis are well-known, and thus implied on their high price in the market. The opposite sides of this, these compounds are similarly difficult to decompose or degrade. Studies shown residues of pharmaceutical and diagnostic compounds have been detected in effluents of sewage treatment plants, surface, ground and drinking waters. Ternes & Hirsch (2000) reported the occurrence of iodinated X-ray contrast media derive from radiological examinations (diatrizoate, iopamidol, iopromide and iomeprol) in sewage treatment plant effluent. Similar study on 1997, Ternes, T.A. reported the occurrence of 32 drugs residues in German municipal sewage treatment plant discharge, river and stream waters. When applying pharmaceuticals and diagnostic agents to humans, a good part of the substances reach the municipal sewage system unchanged or as metabolites.

Ternes, T.A. et al (2002) has shown that drugs and diagnostic agents are not removed quantitatively during waste water treatment by current techniques. From the report, the residues (bezafibrate, clofibrac acid, carbamazepine, diclofenac) are un-removable with flocculation and sand filtration only, with ozonation, we can achieve 90% removal of diclofenac and carbamazepine, 50% removal of bezafibrate, clofibrac un-removable, with granular activated carbon followed by ozonation, all residues are effective removal except clofibrac acid. Hence, it is necessary to develop and evaluate water treatment processes with regard to their potential for eliminating pharmaceuticals and diagnostic agents.

Photocatalysed degradation techniques with titanium dioxide have developed to be one of the most remarkable ways for the treatment of water (wastewater or drinking water) containing organic contaminants. The surface of TiO_2 has a very high oxidation potential (3.0eV) compared to conventional oxidizing agents such as chlorine (1.36eV) and ozone (2.07eV), make it capable of breaking down many organic substance (Fujishima A. et. al.: 1997). The strong oxidizing power of the photogenerated holes, together with the chemical inertness and non-toxicity of TiO_2 , has made it an attractive photocatalyst.

SUMMARY OF SELECTED PAPER

DS Bhatkhande, et al (2001) gave a thorough review of the photocatalytic degradation process in tertiary wastewater treatment. . The review covered the mechanism of the process, a summary of different compounds degradable using the process, the control factors on the process such as light source, adsorption, pH, anions, cations, temperature and surface area. In additions, the catalyst reusability and degradation rates for different compounds had been discussed.

Mechanism

Photons of a certain wavelength incident upon the photocatalyst surface, electrons are promoted from the valence band to the conductance band. This leave positive holes on the valence band, which react with the hydroxylated surface to produce OH^* radicals which are the most potent oxidizing agent. If a suitable

scavenger or a surface defect state is available to trap the electron or hole, their recombination is prevented and a subsequent redox reaction may occur. These will degrade the complex organic compounds to simple organic or complete mineralization to CO₂.

Compounds degradable

Almost all types of organic and inorganic substances can be degraded using photocatalysis. Table 1 shows the summary of compounds reported through out the years,

Table 1. Summary of compounds degraded by various researchers using photocatalyst

Groups	Compounds
Apliphatic	Gaseous formaldehyde, Formic acid, CHCl ₃ , CHBr ₃ , CCl ₄ , Chloroform, Dichloromethane, Trichloroethylene, ethanol, 2-propanol, perchloroethylene, dichloroethane, mono-, di- and trichloroacetic acid, MTBE, glycolic acid, citric acid, monochlotophos
Inorganic	AgNO ₃ , HgCl ₃ , CH ₃ HgCl, reduction of Cr(VI) to Cr(III)
Aromatics	Mallic acid, Benzene, Chlorobenzene, Nitrobenzene, Phenol, Toluene, Salicylic acid, Benzoic acid, p-Hydroxybenzoic acid, 2-Chlorophenol, 4-Chlorophenol, 2,4-Dichlorophenol, 3,5-Dichlorophenol, 2,4,6-Dichlorophenol, 2,3,5-Dichlorophenol, Pentachlorophenol, 4-Nitrophenol, Phenoxyacetic acid, 2,4-Dichlorophenoxy acetic acid, Octaphenylcyclo tetrasiloxane
Surfactant dyes	Textile dye reactive Black 5, Commercial azo dyes, Sodium dodecyl sulfate, Sodium dodecyl benzene sulfonate, p-Nonylphenyl poly(oxyethylene) ether, Methylene blue, Rhodamine B, Methyl orange, Fungicide metalaxyl

Effect of light intensity and wavelength

UV light provides the photons required for the electron transfer from valence band to conduction band of the photocatalyst. The energy of a photon is related to its wavelength and the overall energy input is dependent upon the light intensity. A light source of consistent intensity and particular wavelength is desirable. The wavelength and intensity is dependent on the degraded compounds and catalyst dosage.

Effect of adsorption

The degradation of the substance depends on the adsorption of the substance on TiO₂. The substances which are adsorbed strongly degrade faster.

Effect of pH

Adsorption is at maximum near neutral pH. For weakly acidic substance, degradation increases at lower pH. Some substances undergo hydrolysis at alkaline pH causes higher degradation. In alkaline pH, the OH* radical is higher concentration and may cause higher degradation. For substances which dissociate in certain pH range, the degradation rate will increase.

Effect on anions

Chloride anions decreased the degradation rate due to its adsorption to TiO₂ surface at low pH and strong absorption of UV light. Nitrate anions had a negligible effect. Sulfate anions decreased the degradation rate due to adsorption. Carbonate and bicarbonate anions react with OH* radicals thus reduce the degradation rate.

Effect of cations

Fe³⁺ cations increase the degradation rate at concentration lower than 0.5 mmol/dm³ and retarded the process at excessive concentration. The effect of Fe²⁺ cations is similar. Ag⁺ at concentrations of 0.1 mmol/dm³ has similar effect. Cu²⁺ cations increased the degradation rate slightly at low concentration (<0.01 mmol/dm³) and lower the rate at higher concentrations. The presence of cations in general has a detrimental effect due to associated anions and the effect of salt on substrate adsorption.

Effect of temperature

The degradation rate is weakly dependant on temperature due to low decomposition free energy ($\Delta E=8.37$ kJ/mol). Increasing temperature decreases the solubility of O₂ and thus decreases the concentration of photogenerated holes, thus lower the degradation rate.

Effect of surface area

The photocatalytic degradation is affected by adsorption of the substrate on the catalyst, thus higher surface area will promote degradation rate.

Catalyst reusability

The catalyst can be filtered and reuse for several times for most of the substrate. Catalyst saturated with dye substrates is hardly regenerated. Salt will inhibit the catalyst activity and can be restored by washing with water.

Relative rates of degradation

The degradation of organic compounds proceeds through progressive attachment of OH groups. Thus compounds which facilitate the attachment of OH* radical will degrade faster. The benzene substituted with functional groups contain an unshared pair of electrons will degrade from high to low in the sequence of O⁻, NR₂, NHR, OH, OR, NHCOR, OCOR, SR. Groups that lack of unshared electron, the degradation rate will decrease in the sequence of N_R³⁺, NO₂, CN, SO₃H, CHO, COR, COOH, COOR, CONH₂, CCl₃, NH₃⁺. Surfactants with aromatics rings are more easily degraded than those containing only alkyl or alkoxyate groups. The decomposition rates of amphoteric surfactants are slower than cationic and non-ionic surfactants. The decomposition of nitrogen moieties surfactants is lower and in the following order: pyridine ring > secondary amine > tertiary amine > peptide > quaternary amine. Ring contain nitrogen atoms will not degrade completely.

CURRENT AND FUTURE DEVELOPMENT

The area of photocatalysis has been growth explosively during the past ten years, particularly regarding technology applications. The biggest challenge of photocatalytic degradation is the reactor design. Two typical approaches are generally applied: 1. immobilized the TiO₂ to certain medium. 2. Mixed the TiO₂ particles together with wastewater and separate it from effluent after treatment. For the first approach, several studies had been reported. Fujishima A. et. al. (1997) had developed the TiO₂ films on different substrates such as tile and glass for indoor environmental clean-up. Hofstadler, K. et. al. (1994) reported the reactor design of TiO₂ immobilized on fused silica glass fibers for wastewater treatment. The major problem for first approach will be the robustness of the film and high cost of manufacturing. However, for the second approach, the separation of TiO₂ particles has become an important issue. The classical solid-liquid separation process, such as sedimentation of TiO₂ after pH adjustment or the coagulation with flocculants like basic aluminium chloride, are not satisfactory because the sedimentation take too long hours and after the sedimentation step the supernatant must be filtered.

Doll and Frimmer (2005) reported a good approach of combining cross-flow microfiltration with photocatalytic reactor to achieve long-term stability of the photocatalyst activity. The experiment had been carried out in a pilot plant as shown in Figure 1.

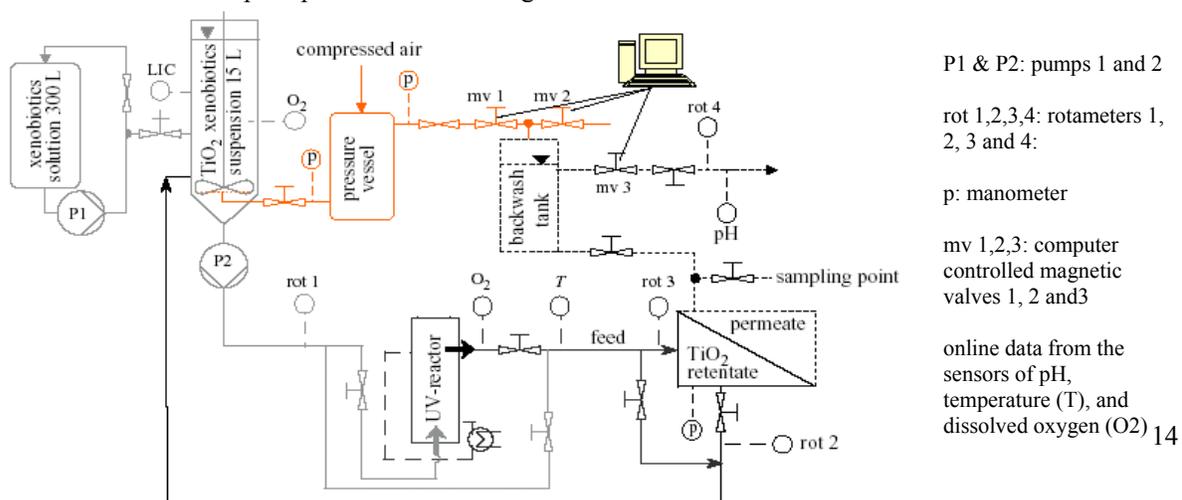


Figure 1. Diagram of pilot plant (Doll & Frimmel :2005)

The combination of TiO₂ with cross-flow microfiltration accompanied by periodical back-wash was investigated in the above pilot plant setup. The investigation included the testing of membrane material because the membrane must resist the abrasion and the periodic back-wash. The potential of two different TiO₂ materials (Hombikat UV100 and Degussa P25) had been evaluated. The investigations showed that the photocatalytic activity of P25 and Hombikat UV100 was constant during continuous usage over several days.

Over the years, studies on the application of photocatalytic degradation process were still in pilot plant or lab scale. More efforts have to be put in to commercialize the process. These should come from researchers' efforts and government's focus on the chronic effects of pharmaceutical and fine chemicals waste.

REFERENCES

- Bhatkhande, D.S., Pangarkar, V.G. and Beenackers, A.A.C.M. 2001. "Photocatalytic degradation for environmental applications – a review". *Journal of Chemical Technology and Biotechnology*. Vol. 77, pp. 102-116.
- Doll, T.E., and Frimmel, F.H. 2005. "Cross-flow microfiltration with periodical back-washing for photocatalytic degradation of pharmaceutical and diagnostic residues – evaluation of the long-term stability of the photocatalytic activity of TiO₂". *Water Research*. Vol. 39, pp. 847-854.
- Fujishima, A. and Rao, T.N. 1997. "Recent advanced in heterogeneous TiO₂ photocatalysis". *Proceedings – Chemical Science (Indian academic Science)*. Vol. 109, No. 6, pp. 471-486.
- Hofstadler, K., Bauer, R., Novalic, S. and Heisler, G. 1994. "New reactor design for photocatalytic wastewater treatment with TiO₂ immobilized on fused silica glass fibers: photomineralization of 4-chlorophenol". *Environmental Science and Technology*. Vol. 28, pp. 670-674.
- Ternes, T.A. 1998. "Occurrence of drugs in German sewage treatment plants and rivers". *Water Research*. Vol. 32, pp. 3245-3260.
- Ternes, T.A., and Hirsch, R. 2000. "Occurrence and behavior of X-ray contrast media in sewage facilities and the aquatic environment". *Environmental Science and Technology*. Vol. 34, pp. 2741-2748.
- Ternes, T.A., Meisenheimer, M., McDowell, D., Sacher, F., Brauch, H.-J., Haist-Gulde, B., Preuss, G., Wilme, U., Zulei-Seibert, N. 2002. "Removal of pharmaceuticals during drinking water treatment". *Environmental Science and Technology*. Vol. 36, pp. 3855-3863.

Enzyme Mediated Asymmetric Synthesis

“Kinetic Resolution and Chemoenzymatic Dynamic Kinetic Resolution of Functionalized γ -Hydroxy Amides”

Prepared by: Lee Siang Hua (HT033441Y)
 Prepared for: Assoc. Prof. Marc Garland
 Date of submission: 13 April 2005

*Dept. of Chemical & Biomolecular Engineering,
 National University of Singapore,
 10, Kent Ridge Crescent, Singapore 119260
 Email: g0302120@nus.edu.sg*

BACKGROUND

The asymmetric synthesis of optically active compounds can be achieved in several ways, 1) resolution of racemates 2) synthesis from the “chiral pool” 3) asymmetric induction using stoichiometric quantity of chiral reagent 4) asymmetric chemical catalysis 5) enzyme-mediated processes. For asymmetric synthesis the most important enzymes are oxidoreductases, hydrolases, lyases (catalyzing additions to double bonds), and less generally, ligases (e.g. aldolases). Some of the enzymes and their functions are listed in Table 1 (compiled from Ullman, Vol. A9, pp. 429-434).

Table 1. Enzymes for Asymmetric Synthesis

Enzymes	Functions	Examples of industrial application
Esterases Amidases	Kinetic resolutions of racemic mixtures	<i>Pig liver esterase</i> - asymmetric synthesis of chrysanthemide, permethrinic, and caronic acids from the corresponding racemic methyl esters <i>Chymotrypsin and acylase</i> – kinetic resolution of amino acids
Lipases Amidases	Hydrolysis	<i>Hog pancreatic lipase</i> – enantioselective hydrolysis of glycidyl butyrate (>95% ee.) <i>Amidases</i> – hydrolyze <i>N</i> -acylamino acids
Amidases	Formation of bonds in polypeptides and proteins	<i>Trypsin-catalyzed reaction</i> – conversion of porcine insulin to human insulin. <i>Thermolysin-catalyzed reaction</i> – synthesis of an aspartame precursor <i>Chymotrypsin, papain and trypsin</i> – total synthesis of dynorphin (an oligopeptide)
Aldolases	Catalyze the cleavage and formation of carbon-carbon bonds in carbohydrates	<i>Fructose 1,6-diphosphate aldolase</i> – synthesis of rare, non-natural and isotopically labeled carbohydrates such as D-Fructose 6-Phosphate and L-Sorbose.
Lyases Hydrolases Isomerase	Catalyzing additions to double bonds Isomerization	<i>α-amylase and glucamylase</i> – conversion of starch to glucose <i>Glucose isomerase</i> – isomerization of glucose to fructose <i>Aspartase</i> – production of aspartic acid <i>Fumarase</i> – production of malic acid from fumaric acid <i>Galactosidase</i> – synthesis of glycosides <i>Epoxy hydrolases</i> – open epoxides regiospecifically
Dehydrogenase Reductase	reduction	<i>Horse liver alcohol dehydrogenase</i> – stereoselective reduction of ketone <i>Enoate reductase</i> – stereoselective reduction of α,β -unsaturated carbonyl compound to the saturated derivatives <i>Lactate dehydrogenase</i> – reduced α -oxo acids enantiospecifically to α -hydroxy acids

The utilization of enzymes in organic synthesis can be advantageous for several reasons (Roberts et al: 1995). Firstly, enzymes catalyze reactions under mild conditions (37°C/1 atm/pH 7). The transformations are often remarkably energy-efficient compared to chemical processes. Secondly, enzymes often promote highly chemoselective, regioselective, and stereoselective reactions, and being chiral catalysts, they are

often able to generate optically active compounds. The increased awareness regarding the need to have optically pure compounds for such uses as pharmaceutical and agrichemicals (so as to avoid unnecessary toxicity and/or ecological damage) has been a significant driving force in the development and exploitation of non-natural enzyme-catalyzed reactions. Thirdly, enzymes can promote reactions that are difficult or impossible to emulate using other techniques of synthetic organic chemistry. Thus bioconversions can generate new series of chiral synthons and, in other cases, may allow a short cut to be taken in a known synthetic sequence. Fourthly, enzymes are natural catalysts, and this can be advantageous where “green appeal” is commercial benefit and/or ecological requirement. Finally, enzymes use water as the reaction medium, and as the disposal of organic solvents becomes progressively more difficult, this may become increasingly important. On the other hand, some enzymes (e.g. lipases) can function as catalysts in organic media, thus allowing a choice to be made regarding the preferred solvent system.

SUMMARY OF SELECTED PAPER

One important function of enzyme in asymmetric synthesis is enzyme resolution of racemic mixture to obtain pure enantiomer. The resolution of racemic mixtures has become an increasingly important field in organic chemistry due to the ease of preparing racemic substrates and the number of transformations that can be exploited to easily change one enantiomer in preference to the other. Kinetic resolution has been a tool for chemists for almost 150 years and remains an important transformation for creating enantiopure samples. The one major limitation of this technique is that the maximum theoretical yield is 50% due to the consumption of only one enantiomer. Because of this, the unreacted enantiomer must be racemized and resubmitted to resolution conditions in order to increase this yield. If the racemization can occur concurrently with the kinetic resolution, known as dynamic kinetic resolution, then theoretically 100% of the racemic mixture can be converted to one enantiomer (Keller:2001).

In the selected paper, Fransson et al (2005) performed an efficient kinetic resolution (KR) of racemic γ -hydroxy amides via *Pseudomonas cepacia* lipase (PS-C)-catalyzed transesterification combined with a ruthenium-catalyzed racemization led to a dynamic kinetic resolution (DKR). The synthetic procedure was illustrated by the synthesis of the versatile intermediate γ -lactone (*R*)-5-methyltetrahydrofuran-2-one.

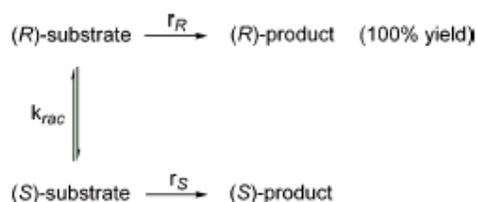
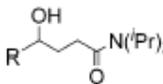


Figure 1. Principle of Dynamic Kinetic Resolution

The basic principle of DKR is illustrated in Figure 1. the un-reactive enantiomer is continuously racemized and the desired enantiomer can be theoretically obtained in 100% yield and 100% ee. In a good DKR process the rate of racemization, k_{rac} , should be higher than the rate of product formation, r_R . The rate of undesired product formation, r_S should be slower than product formation ($r_R \gg r_S$).

The authors had conducted series of experiment to find out the best synthesis conditions, consists of varieties of solvent, acyl donor, hydrogen source, enzymes and derivatives of substrates.

Table 2. Summary of the synthesis

Substrate	γ -hydroxy amides R = Methyl-, Ethyl-, n-propyl-, n-butyl-, NCCCH_2 -, $\text{CH}_2=\text{CHCH}_2$ -, MeOCH_2 -, ClCH_2 - 	The enantioselectivity and the activity decrease with increasing chain.
Enzymes	<i>Candida Antarctica</i> lipase B (CAB), <i>Pseudomonas species</i> lipase (PS-C), <i>Pseudomonas fluorescens</i> (PF), porcine pancreas lipase (PPL), <i>Candida rugosa</i> , <i>Aspergillus</i>	CAB, PS-C and PF showed good activity. CAB show low enantiomeric ratio. PF showed low rate of reaction. No reaction for PPL, <i>C. rugosa</i> and <i>Aspergillus</i> . PS-C showed reasonable activity and excellent enantioselectivity.

Solvent	Toluene, tert-butyl methyl ether (TBME), diisopropyl ether (DIPE), cyclohexane	Ethers and cyclohexane gave a lower enantioselectivity compared to toluene.
Hydrogen source	(1) 2,4-dimethyl-3-pentanol, (2) 2,6-dimethyl-4-heptanol, (3) hydrogen gas, (4) HCOOH and (5) HCOOH.Net ₃ (1:1)	With (4) and (5), slower reaction observed and decreased selectivity. With (1), (2) and (3) formation of ketone is suppressed. Reaction is faster in (3) but (1) appeared to be a better hydrogen donor due to better enantioselectivity.

The kinetic resolution of γ -hydroxy amides (**4**) using PS-C and the acyl donor 4-chlorophenyl acetate (**1**) with a ruthenium-catalyzed racemization process via hydrogen transfer employing the dimeric Ru-precatalyst (**2**) in toluene. The proposed conditions for the dynamic kinetic resolution synthesis process are illustrated using the practical synthesis of the versatile intermediate (*R*)-5-methyltetrahydrofuran-2-one (**6**) as shown in Figure 2. The enantiomerically enriched acetate (**5**) was isolated in 86% yield from *N,N*-diisopropyl-4-hydroxypentanamide (**4**) on a 0.8 mmol scale with an enantioselectivity of 98%. 4-chlorophenyl acetate (**1**) was chosen as the primary acyl donor, 2,4-dimethyl-3-pentanol (**3**) as the hydrogen donor. The optimum performance of the PS-C is about 55-60°C. Acetate (**5**) was transformed to the (*R*)-lactone (**6**) via a one-pot two-step procedure involving hydrolysis with LiOH in methanol followed by acid-catalyzed lactone formation.

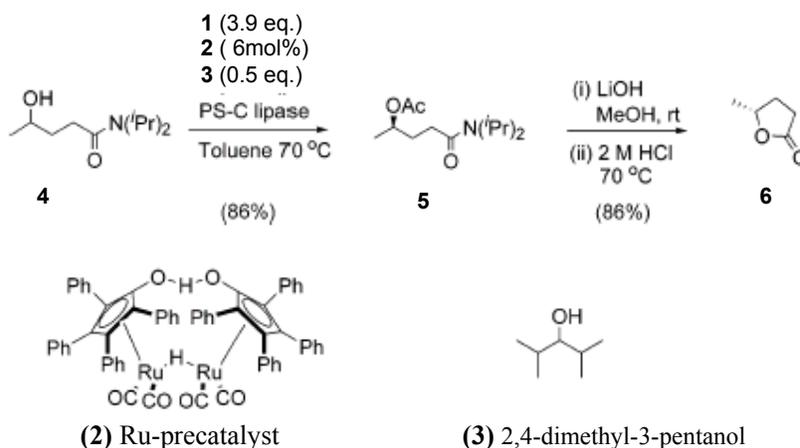


Figure 2. Synthesis of Lactone

CURRENT AND FUTURE DEVELOPMENT

Enzymatic methods will be used increasingly in research and industry for the preparation of chiral compounds. There are wide scopes of research in the enzyme-mediated synthesis. Among those are continually improvement on immobilization techniques, Yashimoto et al (2005) reported a novel immobilized liposomal glucose oxidase system using channel protein OmpF and catalase. The novel biocatalyst gave about 80% of glucose conversion while conventional immobilized glucose oxidase gave only 60% conversion.

Another chemoenzymatic asymmetric synthesis was reported by Jiang et al (2005). The enzymatic resolution of the substrate Boc-aminocyclopenten-1-ol followed by Pd(0) catalysis produced the final product polycyclic pyrazolo[3,4-d]pyrimidine with higher than 90% conversion plus more than 99% ee. The reported process used immobilized enzyme *Candida Antarctica B* (CAB) to enable recycle of enzyme.

Recombinant DNA and RNA techniques are developing rapidly, and with their development, the design and engineering of synthetic catalysts may become feasible. The practice of genetic engineering can now transfer the synthesis of almost any protein into a micro-organism, or some other cell which will grow in a fermenter. The commercial value of the recombined proteins is shown in Table 3 (Roberts et al: 1995).

Table 3. Estimated world-wide sales (1991) of recombinant proteins which are used as pharmaceuticals (Source: Decision Resource Inc)

Recombinant product	Estimated sales (million USD)
Erythropoietin	990
Human insulin	540
Human growth hormone	525
α -Interferon	445
Tissue plasminogen activator	270
Granulocyte colony stimulating factor	255

In addition, enzyme will be useful in the synthesis of complex molecules needed in immunology, endocrinology, intermediate metabolism, molecule genetic, and plant or insect biology. Water-soluble synthetic targets such as carbohydrates and nucleic acids are now commonly manipulated with enzymes. Enzymology complements both classical synthetic chemistry and biological synthetic techniques. Enzymes are also useful in isotopic labeling, analysis and waste treatment.

REFERENCES

- Fransson, A.L., Borén, L., Pàmies, O and Bäckvall, J. 2005. "Kinetic Resolution and Chemoenzymatic Dynamic Kinetic Resolution of Functionalized γ -Hydroxy Amides". *J. Org. Chem.* Vol. 70, pp. 2582-2587.
- Jiang, M.X.W., Warshakoon, N.C. and Miller, M.J. 2005. "Chemoenzymatic Asymmetric Total Synthesis of Phosphodiesterase Inhibitors: Preparations of a Polycyclic Pyrazolo[3,4-d]pyrimidine from an Acylnitroso Diels-Alder Cycloadduct-Derived Aminocyclopentenol". *J. Org. Chem.* Vol. 70, pp. 2824-2827.
- Keller, V. 2001. *Dynamic Kinetic Resolution: Practical Applications in Synthesis* [on-line]. Available from: <http://www.chem.wisc.edu/areas/organic/studsemin/keller/keller-abs.pdf> [Accessed 5 April 2005].
- Roberts, S.M., Turner, N.J., Willetts, A. and Turner, M.K. 1995. *Introduction to Biocatalysis Using Enzymes and Micro-organisms*. US: Cambridge University Press.
- Ullmann's Encyclopedia of Industrial Chemistry, 5th ed. 1987. Germany: VCH
- Yoshimoto, M., Wang, S.Q., Fukunaga, K., Fournier, D., Walde, P., Kuboi, R. and Nakao, K. 2005. "Novel Immobilized Liposomal Glucose Oxidase System Using the Channel Protein OmpF and Catalase". *Biotechnology and Bioengineering*, vol. 90, no. 2, pp. 231-238.