Okolje in farmacevtski izdelki
Prikaz primera: biokataliza L-DOPA

Environment and pharmaceutical products
Case study: Biocatalysis of L-DOPA

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Izvleček

Farmacevtski izdelki so namenjeni zdravljenju in so tako nujni za sodobno družbo. Vendar pa je njihova življenjska doba veliko daljša; ker se v telesu ne absorbirajo ali razgradijo v celoti, predstavljajo problem onesnaženja odpadnih voda. Zdravila in njihove ostanke lahko v izjemno nizkih koncentracijah s postopki sodobne analitične kemije odkrijemo v okolju, in sicer v zemlji in vodi
Kljub nizki koncentraciji so nevarni za žive organizme. Čeprav večine njihovih dolgoročnih učinkov na žive celice ne poznamo, ocenjujejo, da je večina nenevarna. Vseh farmacevtskih izdelkov ne porabimo, veliko zdravil konča v običajnih smeteh, ki tako postanejo nevarne in bi jih morali kot takšne obravnavati. Nevarni odpadki nastanejo tudi pri proizvodnji farmacevtskih izdelkov, zato je cilj čista kemijska sinteza. Med metodami, s katerimi dosegamo ta cilj, je uporaba encimov in

Abstract

Pharmaceuticals cure and treat diseases, and represent the benefit of modern society. However, their lifetime is much longer than many of us are aware. They are not fully absorbed or degraded in the body and their residues are the problem when they enter the sewage treatment systems. Drugs and their residues, in extremely low concentrations, can be detected in the environment: soil and water due to advances in analytical chemistry. Though their concentrations are low, they are a threat to the living organisms. Most of their long-term effects on living cells are unknown but many are known as negative. Not all pharmaceuticals are used; many drugs disappear and end up in the regular domestic waste, which is also a problem, because they present a hazardous waste, and should be treated as such. Dangerous waste is also emerging during the production

celih celic kot katalizatorjev pri kemičnem procesu. Kljub dejstvu, da je uporaba zdravil za človeštvo koristna, pa celoten proces pridobivanja in porabe zdravil zahteva zmanjšanje negativnih učinkov na okolje in živa bitja. Kemiki in inženirji morajo to upoštevati pri razvoju in proizvodnji zdravil.

V članku je opisana uspešna učinkovina L-DOPA, ki je znana kot zdravilo za blažitev znakov Parkinsonove bolezni, njene prednosti in možne nevarnosti. Prikazana je industrijska proizvodnja L-DOPA in tudi njen vpliv na okolje.

of pharmaceuticals. Therefore, the clean chemical synthesis is one of the world's priorities at this time. Among the methods, achieving this goal is the use of enzymes and whole cells as catalysts in chemical processes.

So, if you look at the entire process of production of drugs and their consumption it can be seen that, although the benefits from the use of drugs for the human race beyond doubt, there are a lot of work to reduce their negative side-effects in the environment and living organisms. Chemists and engineers should keep in mind these ideas in the development and production of new drugs, like all of us who use drugs in our favour.

This paper will describe the successful therapeutics – L-DOPA - which is known as a remedy for alleviating the symptoms of Parkinson's disease, and its advantages and potential dangers. Industrial production of L-DOPA will also be discussed, as well as its impact on the environment.

INTRUDUCTION

The impact of pharmaceuticals on the environment

Pharmaceuticals are designed to be resistant to degradation, in order to ensure their appropriate shelf life. This implies that they can not easily be destroyed with simple methods of oxidation, heat treatment, acid or base etc.[1]. Because, pharmaceutical products are now detected in water and soil and represent a danger to the environment. How the new and sensitive analytical methods are developed quickly, these pollutants can be detected in very low concentrations. There is a strong driving force for this development, due to the general concerns about the safety of the environment, water and food. New challenge for the pharmaceutical industry is the production of drugs that will be stable during the manufacturing process, and its shelf life. The metabolism of these drugs in the cells should give inert or environmentally friendly material[1]. It seems like a difficult task, but necessary, because many drugs are a danger to the environment in its original form, as well as conjugates and its metabolites. These compounds can enter the municipal sewage treatment systems, where some of them may be degraded, adsorbed to sewage sludge, or possibly diluted in surface water[2]. It is important to emphasize, if these compounds can not be degraded, then they are hazardous waste streams[3]. People throw into household waste part of the unused medication after the expiration date, and it is also dangerous. Figure 1 shows the methods of entry of drugs into the environment[4]. Because scientists are increasingly aware of the risks to the environment, many are exploring a new ways to design drug. One interesting approach is design of less stable drugs that can be stabilized in formulation matrix[1].

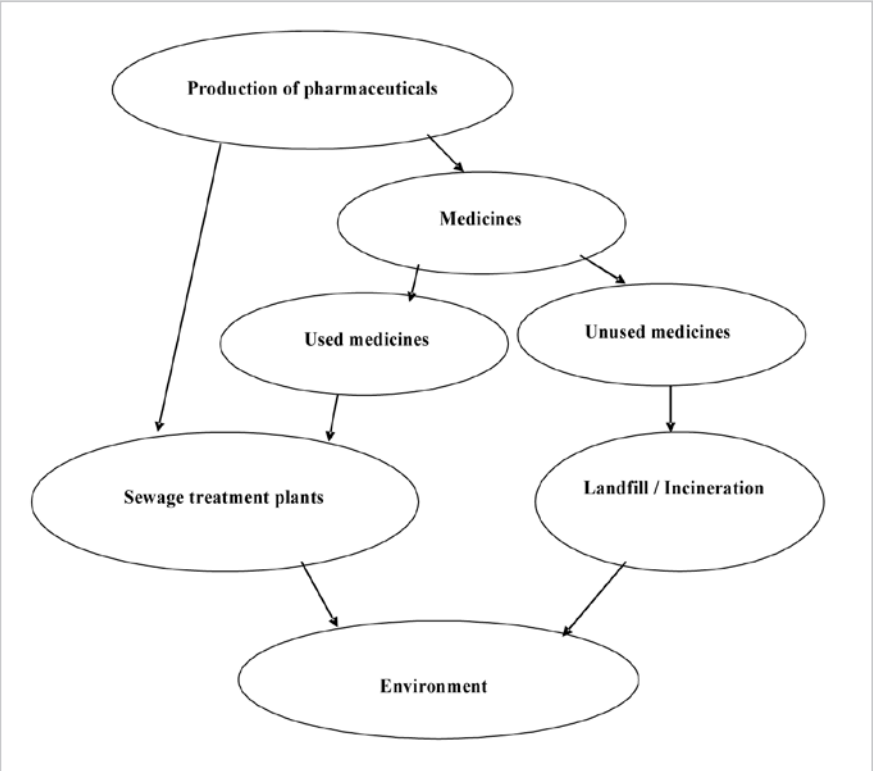

Figure 1. *Entry of medicines into the environment (adapted from ref 4)*

The impact of the chemical industry on the environment

Chemical industry improves the quality of our lives[5]. However, the public image of her is not recognized as a positive, because it causes pollution. Many of the today's chemical processes are based on the technology that was developed in the first half of the 20th century, and because there is an urgent need to develop, new processes that will reduce the amount of waste created. Waste management is expensive, so it has become an important issue for the development of new processes. In addition, recent legislation of European countries includes strict laws against environmental pollution[5] and sustainable development has become a necessary goal in today's society. Therefore, process chemists and chemical engineers should work on new processes that generate less waste, for waste reduction at the end of today's manufacturing process is not sufficient. Processes with higher yields and lower unit operation, should be developed to reduce the environmental quotient[6]. Development should follow twelve principles of green chemistry [7].

Effect of the chemical process on the environment is referred to as *E*-factor, which represents the amount of waste per kg of product[8]. This number indicates the process as environmental friendly or unfriendly. It also reflects the complexity of the synthesis[9]. The *E*-factor is the highest for the pharmaceutical industry, and the lowest for the production of bulk chemicals. This is expected because the production of drugs involves multi-step organic syntheses of non catalytic process. It seems organic chemists are unwilling to accept alternative technologies[8] such as biocatalysis. Since biocatalysis is highly selective and, moreover, able to avoid protective steps, number of steps in the synthesis is often reduced when biocatalysts are introduced, which significantly reduces the high *E*-factor[9]. In the production of chemicals in bulk, traditional environmentally unfriendly processes are largely already been replaced with cleaner, alternative catalytic processes[8]. It is necessary to consider bio catalytic and catalytic processes to improve the *E*-factor, as well as to develop a one-pot multi-step biocatalytic process. Using a one-pot reactions, chemical products and drugs that add value to our lives can be manufactured with less waste and greater economic benefits[10]. By definition, low *E*-factors indicate greener processes. Woodley has presented the advantage of biocatalysis in creation of new green chemical processes.

Chemical vs. biochemical synthesis

Chemical industry serves almost every sector of the economy and the manufacturing, and it is often called the primary industry. Contrary to the chemical industry, bio-industry is not high-volume sales industry but strongly affects the pharmaceutical sector[11]. To improve the sustainability of production, chemical industry is slowly adopting biotechnology as a potential solution. There are many reasons for this; chemical specificity and considerable selectivity (substrate selectivity, regioselectivity, stereoselectivity and functional group selectivity), which inevitably leads to the formation of small amounts of by-products, less waste and cleaner products[12]. The importance of enantiomerically pure drugs has been recognized because it is known that the human body works with chiral catalysis. The main reason for the use of biocatalysis for the production of enantiomers is exploiting its regio- and stereo-selective properties[13]. Enantiomerically pure compounds are often used as intermediates in pharmaceutical manufacturing, for the synthesis of biologically active compounds in drugs. Another advantage of biocatalysis is mild conditions required for the enzyme catalyzed process, which reduces the cost of energy in the process. Enzymes themselves do not pose a threat to the environment because they are completely biodegradable. Their high catalytic efficiency and high yields have been identified in many industrial processes[14]. These are important features of the enzymes that lead to commercially important use in a biocatalytic processes. Despite the many advantages that have enzymes, they are still insufficiently used in technology [12]. The process is economically viable, when the concentration of the product is at least 50-100 g dm^{-3} and 1000 g product per gram of enzyme or unit of enzyme activity [13] is produced, which is not always easy to achieve in the bioprocess.

Case study: L-DOPA

Compound that will be described in the following section is enantiomerically pure drug called L-DOPA. 3,4-dihydroxyphenyl-L-alanine or L-DOPA is a well known pharmaceutical compound that is used to treat of Parkinson's disease, neuro degenerative disorder that affects the elderly population. Guggenheim[15] is the first to isolate L-DOPA from seedlings of *Vicia faba* in 1913. After only two years, the first laboratory synthesis of racemate was published[15]. Guggenheim is assumed that L-DOPA is a precursor in the biological pathway to adrenaline[15,17,18]. After the chemical has given himself, he concluded that it is not biologically active substance and, therefore, took 15 years to establish the contrary. It was found that giving the drug L-DOPA is a way to re-establish the cerebral concentration of dopamine that is deficient in people suffering from Parkinson's disease. Biological activity of L-DOPA was discovered in the early 1960s, and since then there is a growing need for this drug. As a result, many have reported their methods of its synthesis[20]. Historical profile of L-DOPA's can be found in an article in which one described the particular importance of this compound[21].

DISCUSSION

L-DOPA derivatives an its metabolism

L-DOPA, dietetic natural amino acid is an intermediate in several metabolic pathways, and the precursor of catecholamine neuro-transmitters and hormones, as well as melanine[22] and adrenaline[18] (Figure 2. and 3). This compound is fairly unstable in the solution and prone to autooxidation which leads to the development of different oxidation products; *o*-quinone-like compounds which can be easily isolated from the reaction mixture, and can be easily polymerized into melanin in the presence of tyrosinase[23,24]. The oxidation occurs in the presence of oxygen and light and yields toxic free radicals[25]. Without the catalyst, DOPA is oxidized with the reaction rate constant[23] of approximately 10^{-7} s. Except for their instability, L-DOPA is also known for its poor solubility in water, which makes its separation from the reaction solution easier[26]. It is precipitated and can be collected by centrifuga-

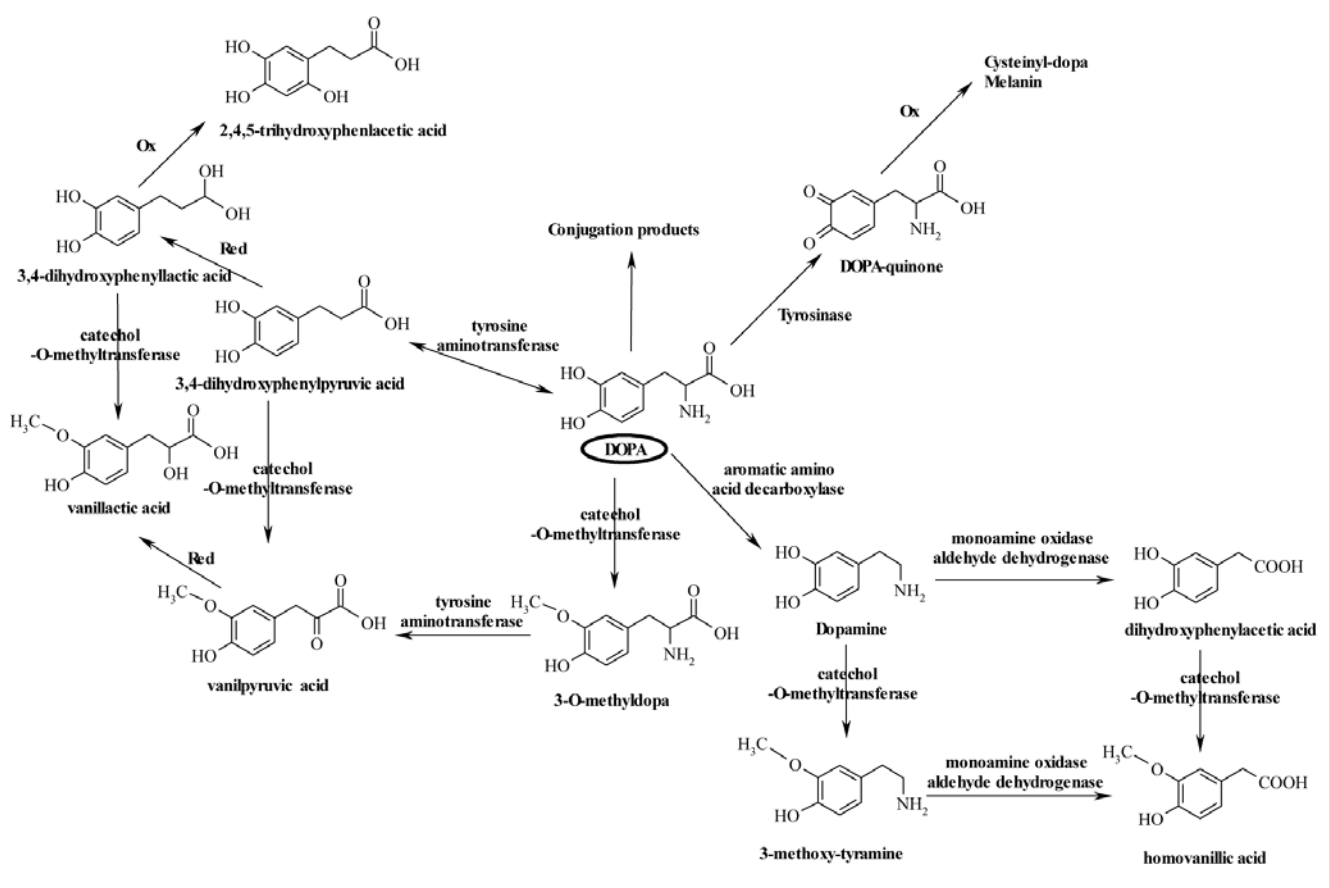

Figure 2. *L-DOPA metabolism (adapted from ref 22).*

tion[27]. Thus, L-DOPA is a threat to the environment and therefore its transformation into less toxic products is desirable.

There are many important derivatives of L-DOPA, which are known to have biological activities and are less harmful to the environment. One of them is an important traditional medicine called *Danshensu* or 3,4-dihydroxyphenyllactic acid. It can be synthesized by oxidative deamination of L-DOPA catalyzed by L-amino acid oxidase (Figure 4)[28] followed by reduction of product (3,4-dihydroxyphenylpyruvic acid) to 3,4-dihydroxyphenyllactic acid that is catalysed by the D-lactate dehydrogenase[29]. 3,4-dihydroxyphenyllactic acid is used to treat

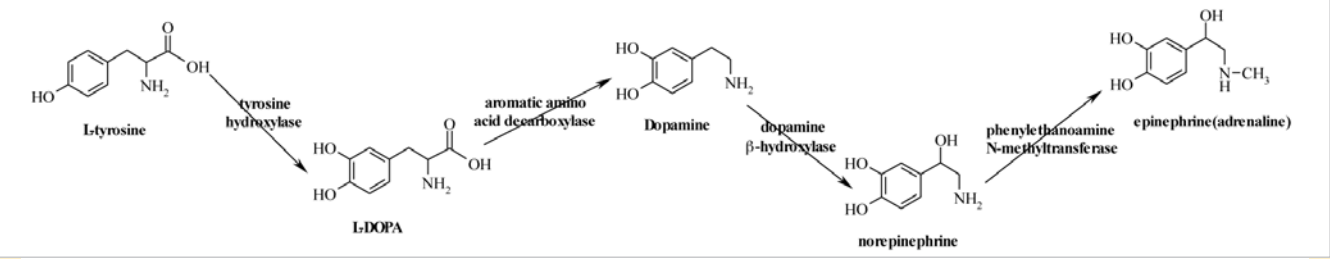

Figure 3. *Pathway of L-DOPA to adrenaline.*

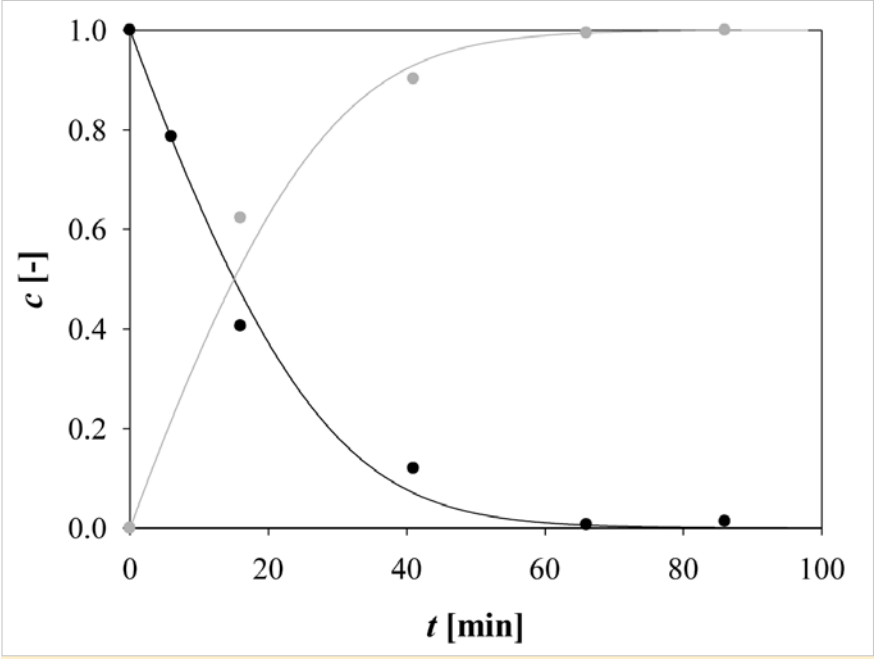

Figure 4. *Enzymatic oxidation of L-DOPA ($c_{cL\text{-}DOPA,0}$ = 3.83 mmol dm^{-3}) with L-AAO from C.adamanteus ($c_{cL\text{-}AAO}$ = 0.308 U cm^{-3}) in phosphate buffer (0.2 mol dm^{-3}, pH 7.8) in batch EMR ($c_{catalase}$ = 8790.9 U cm^{-3}) (●L-DOPA, ●3,4-Dihydroxyphenylpyruvic acid, ___ model) (adapted from ref 28).*

menstrual disorder, menostasis, menorrhalgia, insomnia, diseases of blood circulation and angina pectoris[30]. Another interesting derivative of L-DOPA, which is closely associated with the 3,4-dihydroxyphenyllactic acid, was rosmarinic acid. This is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid and is known for its biological activity, including antiviral, antibacterial, anti-inflammatory and antioxidant[31]. Its chemo-enzymatic synthesis has been reported[32].

L-DOPA synthesis

The first papers, which describe the synthesis of L-DOPA [33, 34], are from the 1969th. L-DOPA, as a possible cure for Parkinson's disease, has already attracted

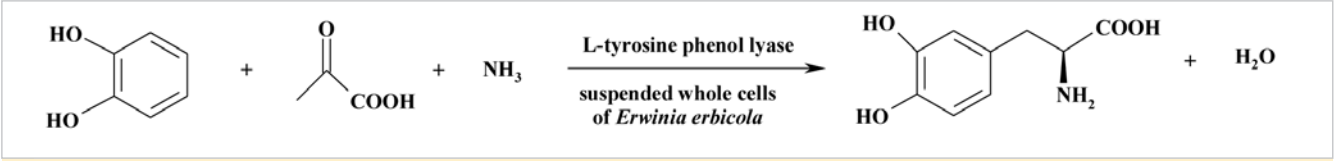

Figure 5. *Enzymatic synthesis of L-DOPA by Ajinomoto Co. Ltd.*

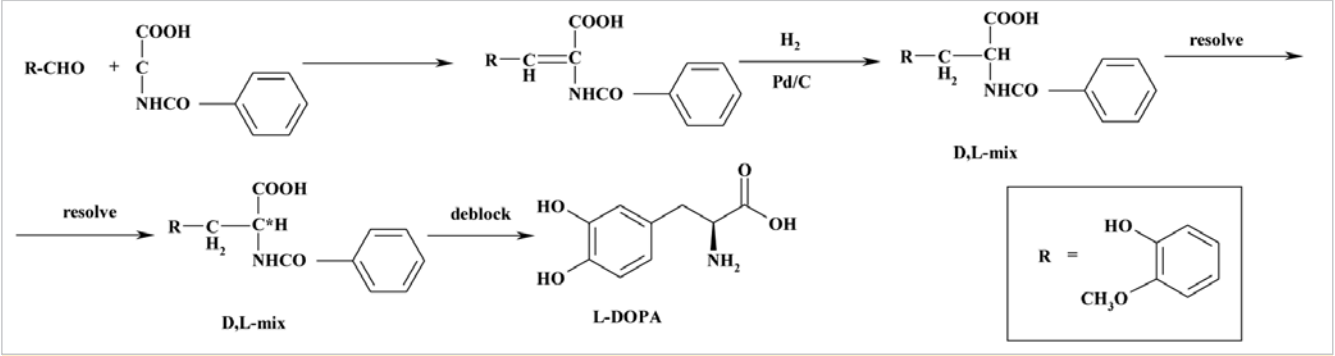

Figure 6. *Chemical synthesis of L-DOPA by Hoffman-La Roche.*

much attention [35]. Haneda et al[35] reported the synthesis of L-DOPA from L-tyrosine in one-step oxidation catalyzed by tyrosinase or tyrosine hydroxylase, which is a part of the natural pathway from L-tyrosine to melanine and catecholamines. There are numerous reports on L-DOPA synthesis since than, because of their importance for the pharmaceutical industry. Enei et al. synthesized L-DOPA from pyruvate, ammonia and pyrocatechol[36, 37]. Yoshida et al. produced L-DOPA with the bacterium *Vibrio tyrosinaticus* and *Pseudomonas melanogenum*[38-40]. Para and Baratti used immobilized cells of *Erwinia herbicola* (tyrosine phenol lyase) for L-DOPA synthesis[41]. Many other authors have worked on L-DOPA synthesis[24, 27, 42-47].

L-DOPA is now industrially produced compound by several pharmaceutical companies. Both biocatalytic and chemical synthesis methods are used. Company Ajinomoto Co. Ltd. produces L-DOPA with enzymatic synthesis[14] in fed-batch reactor by a reaction scheme, which is shown in the Figure 5. Synthesis is carried out using whole cells of *Erwinia erbicola* containing L-tyrosine phenol lyase as a biocatalyst. Process (Figure 5) is relatively simple compared to chemical methods (Figure 6 and 7). There is only one reaction step, and next to the desired product, no other by-products exist except for water. Whole cells can be easily separated from the reaction solution after the reaction ended. Toxic substrates are fed to the reactor and there is no need for the excess of toxic chemicals. Therefore, this method has lower environmental impact than chemical methods that have a large number of intermediate steps (Figure 6 and 7). The industrial chemical process for the synthesis of L-DOPA has been developed by Hoffman-La Roche in 1967, and the reaction scheme is shown in Figure 6. William S. Knowles had developed the industrial process for production of L-DOPA for Monsanto, which is based on catalytic asymmetric hydrogenation year later[48]. Reaction scheme is shown in Figure 7. Knowles's discovery was awarded by the Nobel Prize in 2001 (for the catalytic asymmetric hydrogenation with the rhodium metal catalyst) [48]. Monsanto Company has successfully scaled up the chemical synthesis. Comparing the enzymatic (Fig. 5) and chemical synthesis (Figure 6 and 7), can be seen that chemical synthesis is much more complicated, because a number of steps, probably has a higher *E*-factor. After each step of reaction, intermediate product is separated, often in low yield. For the purpose of separation, organic and potentially toxic chemicals are required. Therefore, many chemicals end up in waste water. However, in enzymatic synthesis, there

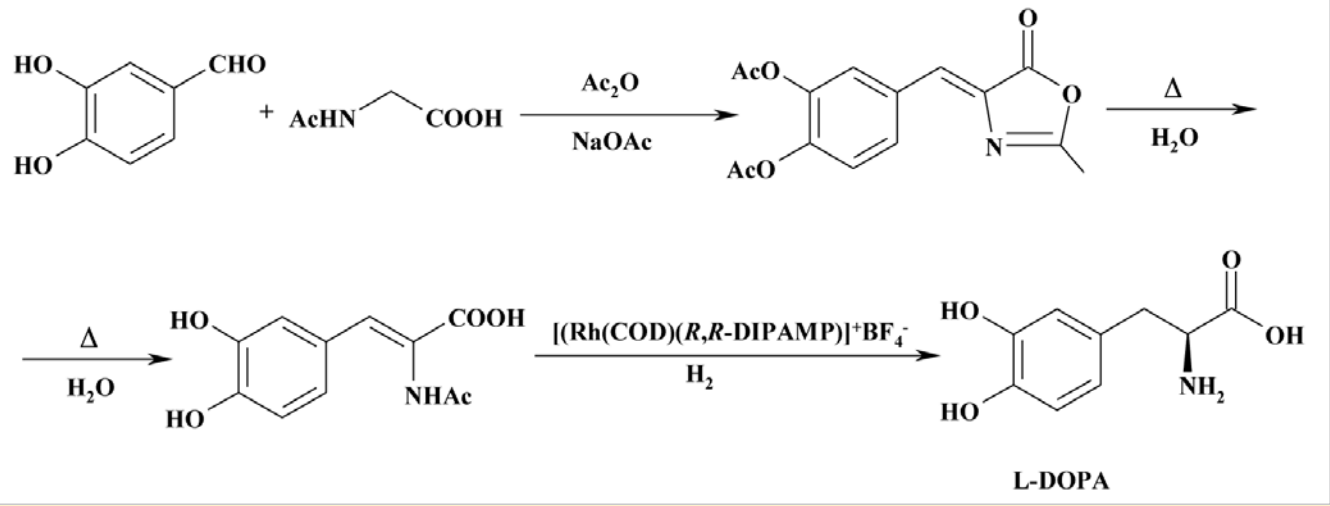

Figure 7. *Chemical synthesis of L-DOPA by Monsanto.*

is only one reaction step that reduces the process time and the consumption of chemicals.

CONCLUSION

New industrial processes that will be developed must be more in terms of green chemistry and environmental protection. This means reducing waste in the process to a minimum, using less complicated synthesis, less organic solvents, safer solvents and reducing energy consumption in the process. Since the enzymatic synthesis is usually carried out in mild reaction conditions and does not need toxic chemicals, it is *a priori* method of choice (low energy consumption).

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