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A Practical High Through-Put Continuous Process for the Synthesis of Chiral Cyanohydrins

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Abstract: A practical high through-put continuous process for the synthesis of chiral cyanohydrins is reported. Pretreated almond meal (or other solid raw enzyme sources) was loaded in a column to form a reactor, to which were attached a supplying system to deliver a solution of substrate and HCN in solvent on one end and a collecting−separating system on the other end. By controlling the flowing rate, optimal conditions were achieved for the hydrocyanation of various aromatic carboxaldehydes in a "micro-aqueous" medium to produce chiral cyanohydrins in high yields and high enantiomeric excess (ee) with high substrate/catalyst ratio.

Cyanohydrins not only play an important role at the interface between chemistry and biology, but they also have considerable synthetic potential as chiral building blocks, especially in a wide range of pharmaceutical and agrochemical applications. With regard to the preparation of chiral cyanohydrins, it can be mentioned that, in addition to the asymmetric synthetic methods such as being catalyzed by cyclic peptides or chiral Lewis acid,[1] the biocatalytic hydrocyanations with oxynitrilases provide a competitive access in the formation of (*R*)- and (*S*)-cyanohydrins. This enzymatic approch has been successfully developed since the pioneering work of Effenberger.[2] Application of an almond meal as a solid crude enzyme source[3a] and a polymer-entrapped (*R*)-oxynitrilase as an immobilized enzyme[3b] have been reported in the hydrocyanation reaction in a biphasic (aqueous buffer solution and organic solvent) reaction system.

In the case where enzyme preparation is in the solid form (e.g. the crude enzyme source preparation and the immobilized enzyme as mentioned above), enzymatic reactions can be efficiently performed in a continuous way in a tubular reaction vessel to give a higher through-put than in a batch process. However, reactions in aqueous buffer solution have to be carried out batchwise, because the solid enzyme preparations may clog the reactor due to their swelling by absorption of the water in aqueous medium.

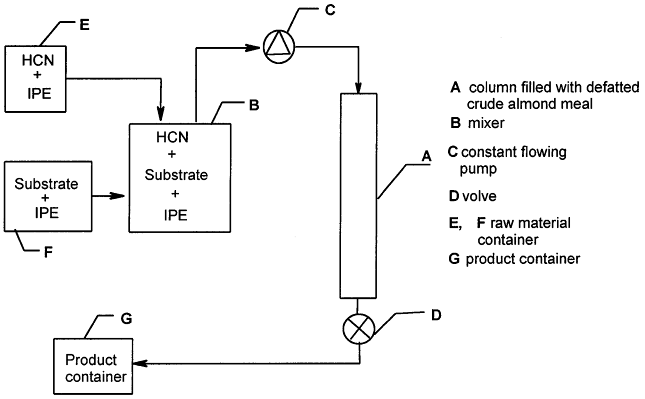

FIGURE 1. A schematic flow sheet of the continuous process.

In the previous papers,[4] we described an efficient catalytic process using a defatted crude almond meal (peach or loquat kernel meal works as well) in a micro-aqueous organic solvent system for the formation of chiral cyanohydrins in high enantioselectivities and high yields. The enzyme was retained in the intact cells and kept as in the natural environments. Furthermore, the organic medium used for the reaction caused no swelling problem. Therefore, we envisioned that this reaction condition could be applied to a column or pipe-like reactor to continuously produce the desired product in high through-put. This would not be applicable to the biphasic system, because the plant cells would absorb the water contained in the system and become swollen, finally clogging the column or pipe reactor.

This process involves (1) preparing meal of an enzyme source material (for example, almond kernel) by soaking the material in water, air-drying, pulverizing the swollen material in a homogenizer, and then defatting the obtained meal, which contains a micro-amount of water (8.96% (w/w)) after the above-mentioned treatment; (2) loading the resultant meal into a column; and (3) carring out the reaction in an organic solvent such as isopropyl ether (IPE, with a water content of 0.3% v/v). Benzaldehyde was hydrocyanated in a column filled with ca. 15 g of the defatted almond meal to produce mandelonitrile in both good yields and enantiomeric excess. (Table 1).

As shown in Table 1, after in total 2 mol of the substrate was treated, the column still retained its high catalytic activity. The through-put of the column is 3630 g/(L·day) (grams of the cyanohydrin per liter of the almond meal in a day). According to van der Gen et al.,[6] two grams of pure oxynitrilase could be obtained from 1 kg of almond kernel. Therefore, the column efficiency can

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(1) For examples, see: Abiko, A.; Wang, G. *J. Org. Chem.* **1996**, *61*, 2264. Hayashi, M.; Miyamoto, Y.; Inoue, T.; Oguni, N. *J. Chem. Soc., Chem. Commun.* **1991**, 1752.

(2) For the development of the enzymatic asymmetric synthesis of cyanohydrins, see: (a) Effenberger, F. *Angew. Chem., Int. Ed.* **1994**, *33*, 1555. (b) Effenberger, F.; Föster, S.; Wajant, H. *Curr. Opin. Biotechnol.* **2001**, 532. (c) Gröger, H. *Adv. Synth. Catal.* **2001**, *343*, 547. (d) Gregory, R. J. H. *Chem. Rev.* **1999**, *99*, 3649.

(3) (a) Huuhtanen, T. T.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1992**, *3*, 1223. (b) Gröger, H.; Capan, E.; Barthuber, A.; Vorlop, K.-D. *Org. Lett.* **2001**, *3*, 1969.

(4) (a) Han, S.; Lin, G.; Li, Z. *Tetrahedron: Asymmetry* **1998**, *9*, 1835. (b) Lin, G.; Han, S.; Li, Z. *Tetrahedron* **1999**, *55*, 3531. (c) Han, S.; Chen, P.; Lin, G.; Huang, H.; Li, Z. *Tetrahedron: Asymmetry* **2001**, *12*, 843. (d) Chen, P.; Han, S.; Lin, G.; Huang, H.; Li, Z. *Tetrahedron: Asymmetry* **2001**, *12*, 3273.

(5) Brussee, J.; Loos, W. T.; Kruse, C. G.; van der Gen, A. *Tetrahedron* **1990**, *46*, 979.

(6) Smitskamp-Wilms, E.; Brussee, J.; van der Gen, A. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 209.

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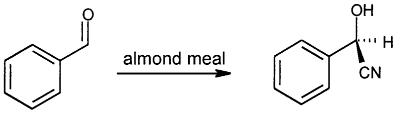
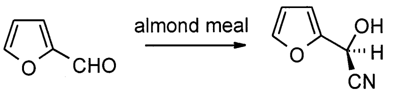
TABLE 1. Hydrocyanation of Benzaldehyde in Column (inside diameter 0.7 cm × length 80 cm, with ca. 15 g of the defatted almond meal, flow rate = 3 mL/min, *T* = 15 °C)



run	substrate (mmol)	total substrate (mmol)	yield (%)	ee (%)[a]
4	10	40	91	99
13	10	130	92	>99
25	10	250	95	>99
32	10	320	94	>99
50	10	500	91	>99
110	10	110	89	>99
150	10	1500	90	>99
200	10	2000	89	>99

[a] The enantiomeric excess (ee) was obtained by comparing the determined specific optical rotation to the reported data.[5]

TABLE 2. Hydrocyanation of Furanyl-2-carboxaldehyde (flow rate = 1 mL/1 min, *T* = 15 °C)



run	substrate (mmol)	total substrate (mmol)	yield (%)	ee (%)[a]
1	10	10	94	99
12	10	120	93	98
17	10	170	99	99
24	10	240	98	99
30	10	300	100	99
38	10	380	95	99
50	10	500	98	98
60	10	600	97	97
90	10	900	94	97
120	10	1200	96	98

[a] The enantiomeric excess (ee) was obtained by comparing the determined specific optical rotation to the reported data.[7]

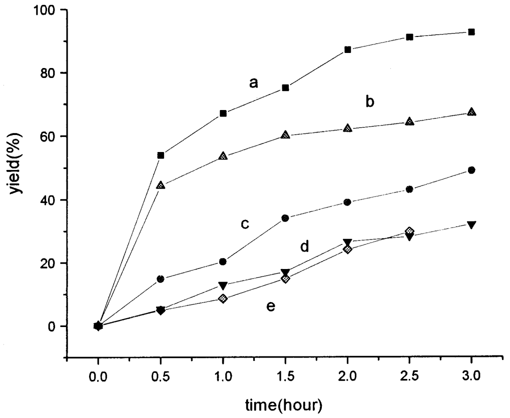
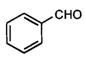
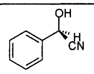
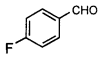
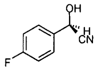
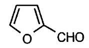
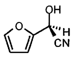
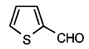
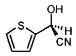
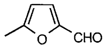
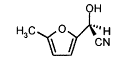


FIGURE 2. Time courses of cyanohydrins for five aldehydes: (a) benzaldehyde, (b) *p*-fluorobenzaldehyde, (c) furanyl-2-carboxaldehyde, (d) thienyl-2-carboxaldehyde, and (e) 5-methylfuranyl-2-carboxaldehyde.

TABLE 3. Hydrocyanation of Five Heteroaryl Carboxaldehydes in Column (inside diameter 0.7 cm length 80 cm, with ca. 15 g of the defatted almond meal, *T* = 15 °C)

Substrate	Flow rate (mL/min)	Product	Yield (%)	*e.e.* (%)	Config.
	3	 **1**	95	>99[c]	*R*
	2	 **2**	92	84[d]	*R*
	1	 **3**	100	>99[e]	*S*[a]
	1	 **4**	70[b]	99[e]	*S*[a]
	1	 **5**	99	97[f]	*S*[a]

[a] The *S* configurations are due to the Cahn–Ingold–Prelog rules; no reversal of the absolute configuration on the chiral carbon occurs. [b] Calculated based on the starting material used. The reaction mixture collected from the column reactor contained the cyanohydrin as a sole product, no starting material remained. [c–f] The enantiomeric excesse (ee) was obtained by comparing the determined specific optical rotations to the reported data.[4c,5,7,8] For details, see the Experimental Section.

be described by S/C = 66.7 mol/g (moles of the substrate/gram of the pure enzyme protein).

Hydrocyanation of furanyl-2-carboxaldehyde performed under the same conditions but with slower flow rate also gave sastisfactory results (Table 2). The through-put is calculated to be 1176 g/(L·day) and S/C = 40 mol/g.

In most cases, the resulting cyanohydrins are used as intermediates for further reaction. The yields and ee values shown in the above tables indicate that the products have sufficient purity for further reactions. No purification operation is needed.

In our experiments, it is found that higher flow rate favors the enhancement of the enantiomeric excess but compromises the chemical yields. Because different aldehydes undergo hydrocyanation in different reaction rates, it is necessary to find an optimal flow rate suitable for individual substrate to achieve a balance between the yield and the enantiomeric excess. For this purpose, five aldehydes were subjected to hydrocyanation in separate flasks under the same conditions. Figure 2 shows the time-course of the reaction.

The relative reaction rates for substrates (a, b, c, d, and e, respectively) were calculated according to the following equation to be a:b:c:d:e = 100:66:44:28:22. Therefore, the flow rate for these individual substrates was set in accordance with their relative reaction rates as shown in Table 3.

$$\text{flow rate (mL/min)} = 3 \times \frac{\text{relative reaction rate of aldehyde}}{\text{relative reaction rate of benzaldehyde}}$$

In conclusion, by taking advantage of the unique feature of the defatted almond meal in the micro-aqueous medium,[4] we are able to establish a continuous process for asymmetric hydrocyanation with high yields and ee values in high through-put. No purification operation is needed in this process. Although the preparation of five chiral cyanohydrins is described for illustration of the efficiency and scope of the method, more cyanohydrins can be prepared with the technique by adjusting the column length, the reaction temperature, and the flow rate.

Experimental Section

Caution: HCN is extremely toxic and must be handled with high caution. Safety instruction is given in the Material Safety Data Sheet (MSDS) of HCN. For further safety information, see the International Chemical Safety Card of HCN (ICSC0492), available from http://www.cdc.gov/niosh/ipcsneng/neng0492.html.

General. Almond kernels were soaked in distilled water for 2 h, peeled, air-dried, and then powdered in cold ethyl acetate with a homogenizer. The powder was defatted by a further three washes with ethyl acetete, filtered, and stored in a refrigerator. After 1 g of sea sand was added to a glass column with a sintered glass frit, the column was loaded with approximately 15 g of the crude almond meal powder. Finally, 1 g of sea sand was added to complete the preparation of the enzyme column. A solution of 10 mmol of the substrate in 50 mL of IPE and a solution of HCN (1.5 equiv)/IPE were mixed and the mixture was pumped passing through the enzyme column under the reaction conditions mentioned in the above tables. The eluate was pooled and extracted with saturated aqueous ferric chloride solution in a separatory funnel for several times to remove the residual HCN until the brown color of ferric chloride solution no longer changes to blue. The organic phase was worked up in the conventional manner (washed with water, dried over anhydrous Na_2SO_4, and concentrated in vacuo) to give the product.

Mandelonitrile (1): clear oil; yield 95%; $[\alpha]^{20}_{D}$ +47.5 (*c* 1.89, $CHCl_3$), ee >99% (lit.[5] [α] +45 (*c* 1, $CHCl_3$), ee >99%); ^1H NMR δ 3.70 (s, 1H, OH), 5.50 (s, 1H, CH), 7.50 (m, 5H, Ar−H).

4-Fluoromandelonitrile (2): clear oil; yield 92%; $[\alpha]^{20}_{D}$ +32.6 (*c* 6.8, $CHCl_3$), ee 84% (lit.[4c] [α] +36.4 (*c* 6.38, $CHCl_3$), ee 94.2%); ^1H NMR ($CDCl_3$) δ 3.39 (br, s, 1H, OH), 5.52 (s, 1H, CH), 7.13 (m, 2H, Ar−H), 7.49 (m, 2H, Ar−H) ppm; ^{19}F NMR ($CDCl_3$/TFA, ppm) −33.93 (m, Ar−F).

(*S*)-(+)-2-Hydroxy-2-(2-furyl)acetonitrile (3): clear oil; yield 100%; $[\alpha]^{21}_{D}$ +50.0 (*c* 1.60, $CHCl_3$), ee >99% (lit.[7] [α] +14 (*c* 1.87, $CHCl_3$), ee 79%); ^1H NMR ($CDCl_3$) δ 3.17 (br, s, 1H, OH), 5.55 (s, 1H, CH), 6.43 (dd, 1H, J_1 = 1.9 Hz, J_2 = 3 Hz, Ar−H), 6.61 (m, 1H, Ar−H), 7.49 (m, 1H, Ar−H) ppm.

(*S*)-(+)-2-Hydroxy-2-(2-thienyl)acetonitrile (4): clear oil; yield 70%; $[\alpha]^{21}_{D}$ +62.4 (*c* 2.3, $CHCl_3$), ee >99% (lit.[7] [α] +61.6 (*c* 1.6, $CHCl_3$), ee 99%); ^1H NMR ($CDCl_3$) δ 3.0 (s, 1H, OH), 5.8 (s, 1H, CH), 6.9−7.4 (m, 3H, Ar−H).

(*S*)-(+)-2-Hydroxy-2-(5-methyl-2-furyl)acetonitrile (5): clear oil; yield 99%; $[\alpha]^{21}_{D}$ +60.1 (*c* 1.75, $CHCl_3$), ee 97% (lit.[8] [α] +61.0 (*c* 1.60, $CHCl_3$), ee 99%); ^1H NMR ($CDCl_3$) δ 2.5 (s, 3H, CH_3), 3.0 (s, 1H, OH), 5.5 (s, 1H, CH), 6.0−7.2 (m, 2H, Ar−H) ppm.

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[7] (a) Effenberger, F.; Ziegler, T.; Föster, S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 458. (b) Effenberger, F.; Eichhorn, J. *Tetrahedron: Asymmetry* **1997**, *8*, 469.

[8] Zandbergen, P.; van der Linden, J.; Brussee, J.; van der Gen, A. *Synth. Commun.* **1991**, *21*,1387.