Racemic Leucine Separation by Hollow-Fiber Extraction

H. B. Ding, P. W. Carr, and E. L. Cussler*
Dept. of Chemistry, Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN 55455

Racemic leucine can be separated into d- and l-isomers by fractional extraction across microporous hollow fibers. In this extraction, an aqueous solution of the racemate is fed to the lumen of the fibers, and an octanol solution of dodecyl-l-hydroxyproline flows countercurrently outside of the fibers. The interface between feed and extractant is stabilized by filling the pores in the hollow-fiber walls with a cross-linked polyvinylalcohol gel which offers negligible resistance to mass transfer. The extraction with dodecyl-l-hydroxyproline deliberately imitates earlier studies, facilitating comparisons of hollow-fiber extraction with other techniques. The results show that the isomer yield per equipment volume of racemic separation is 100 times greater than that in a continuously rotating extractor, and 1,000 times greater than that in a conventional packed tower.

Introduction

This article demonstrates the separation of racemic leucine by hollow-fiber-based liquid extraction. Hollow fibers are an increasingly attractive alternative to conventional packed towers for gas adsorption and for liquid-liquid extractions (Prasad and Sirkar, 1990; Ding and Cussler, 1991). The attraction of hollow fibers does not result from large mass-transfer coefficients; indeed, their mass-transfer coefficients are similar to those in conventional equipment. Instead, the attraction of hollow fibers results from their unusually high surface area per volume. This surface area per volume is typically 30 times larger for fibers than for packed towers, and 100 times larger for fibers than for conventional extractors (Dahuron and Cussler, 1988).

Of course, these advantages of hollow fibers can be lost if the fibers themselves are impermeable, and hence dramatically reduce mass-transfer rates. To avoid this, many previous workers have used microporous, hollow fibers. Because these fibers are microporous, they permit easy diffusion between the two fluid phases. Sometimes, however, that fluid phase which wets the hollow fiber leaks through the porous fiber wall and is entrained as a foam or an emulsion which often compromises the separation. Previous researchers avoid this in two ways. First, they apply a static pressure to the phase that wets the membrane less easily (Prasad and Sirkar, 1990). This static pressure, often around 1 atm, pushes the leaking phase back into the pores and thus prevents entrainment. The second way to keep the phases separate but the pores free is to coat the hollow fiber with a highly permeable polymer or gel (Schisla, 1990; Ding and Cussler, 1991). This polymer or gel stops flow through the pores, but allows diffusion to occur without check. In these ways, the fast separations possible with hollow fibers are easily realized.

In this article, we explore the difficult separations of chiral mixtures as an example of hollow-fiber extraction. These separations are important because chiral mixtures include many pharmaceuticals, flavors, insecticides, herbicides, and pheromones. Only one enantiomer in the chiral mixture produces the desired effect; sometimes the other enantiomer causes undesirable side effects. However, chiral separations are difficult, because the enantiomers have identical physical and chemical properties.

The earliest chiral separation, due to Pasteur (1848), is by fractional crystallization. Pasteur crystallized sodium ammonium tartrate from aqueous solution and then separated the crystals by hand—for six months. Modern methods depend on crystallizers, but are limited to cases where the solubility of individual isomers is less than the solubility of the racemate. Diasteromer formation, differential enzyme reactivity, and adduct formation with species like urea and the cyclodextrins have also been used on a regular basis. All these methods may

*E. L. Cussler performed work on this article while at the Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139.

require many stages, for each usually gives only a small separation per stage (Addadi et al., 1982).

The chief competition to these classical methods is liquid chromatography (Ahuja, 1991). This method uses a bed packed with spheres which may be as small as 5 μm in diameter. These spheres are coated or filled with optically active liquids. A pulse of solution of enantiometers is forced through the packed bed, and different enantiomers are adsorbed differently. The adsorbed complexes are essentially short-lived diasteromers connected by more than one bond to sites close to the asymmetric site, which is usually a carbon atom. Obviously, the separation depends critically on the adsorbed sites, which are intellectually exotic, chemically intriguing, and expensive. Still, while chromatography can separate more mixtures than crystallization and the other classical methods, it is hard to operate at an industrially significant scale.

Extraction with an optically active extractant is an attractive option. While the selectivity of a single extraction is small, use of extractions with many stages is well established. The capacity of extraction is often much greater than that of chromatography. Differential extraction can easily be operated continuously. We thought that chiral extraction in hollow fibers might have special promise, which is explored here.

The particular system chosen to be studied is based closely on work of Takeuchi et al. (1984). These authors used a feed of racemic amino acids dissolved in water. They used an extractant of an alkylhydroxyproline and cupric ion dissolved in *n*-butanol and other solvents. The alkyl chain was most effective if it was C_8 or higher; the cupric ion was the center of chelation. Takeuchi et al. (1990) used this system to resolve racemic leucine by fractional extraction in rotating segmented glass columns. Thus, by choosing this system for our experiments, we can compare our hollow fibers with one of the most attractive alternative methods. The comparison begins in the next section.

Experimental Studies

All the chemicals are from Aldrich except as noted. To synthesize *N-n*-dodecyl-*l*-hydroxyproline, 0.15 mole of *n*-dodecyl aldehyde is dissolved in 100-mL absolute ethanol; then 0.1 mol of *l*-hydroxyproline is added to the solution. One and a half grams of 5% palladium on activated carbon is suspended in 50-mL absolute ethanol and added carefully to the reactants. The mixture is stirred with a magnetic stirrer and sparged with hydrogen under ambient pressure and at room temperature for seven days. The completeness of reaction is monitored via the intensity of the *l*-hydroxyproline spot produced by the thin-layer chromatography on alumina TLC plates (Polygram Alox N/UV 254, Macherey-Nagel). After all the hydroxyproline has reacted, the activated carbon is removed by filtration under reduced pressure and washed with ethanol three times. All liquid samples are collected, mixed, and evaporated under vacuum. The residue is washed with ether and recrystallized from water. Mass spectroscopy is consistent with the desired product, and waxy flakes obtained have a melting point of 167 ± 2°C, close to the value of 165-167°C given by Takeuchi et al. (1984). While our synthesis parallels that given in the reference, the reaction conditions given here produced a better yield.

The partition coefficients of the leucine enantiomers are measured by contacting the octanol phase containing *N-n*-dodecyl-*l*-hydroxyproline with the aqueous phase containing *d*-leucine and *l*-leucine. While octanol is basically insoluble in water, it is one of the solvents used by Takeuchi et al. (1990). The octanol phase is prepared by first dissolving 20-mM *N-n*-dodecyl-*l*-hydroxyproline in 1-octanol. The aqueous phase is prepared by dissolving 10-mM copper acetate in 0.2 M-acetate buffer at pH 4.0. The two phases are then mixed in a separatory funnel for about 20 minutes. After the two phases separate, the aqueous phase contains 2 mM of copper (II) and basically no *N-n*-dodecyl-*l*-hydroxyproline. The final octanol phase contains 8 mM of copper (II), 20 mM of *N-n*-dodecyl-*l*-hydroxyproline, and some water. The aqueous leucine is prepared by mixing 5.0 mL of 37.0-mM *dl*-leucine stock solution with 195 mL of the aqueous phase prepared as described above. The final solution contains 0.925-mM *dl*-leucine. Equal volumes of octanol solution and aqueous *dl*-leucine solution are mixed in test tubes for 20 minutes. After the two phases are separated, the concentrations of *d*-leucine and *l*-leucine are measured.

The composition of this new mixture is analyzed by reversed-phase liquid chromatography (Wernick, 1985). The reversed-phase column, packed in our lab with Hypersil MOS 5-μ particles with C-8 bonding, uses as a mobile-phase aqueous chiral buffer containing 2-mM *l*-phenylanaline, 1-mL copper (II), and 0.2-M acetate at pH 4.0. A Milton-Roy variable wavelength UV detector (Model #3010) is used at wavelength of 265 nm. The column is attached to a Spectra-Physics Isochrom digital pump and Rheodyne 7010 injection valve with a 10-μL sample loop.

The hollow-fiber extraction of racemic leucine uses two pairs of two identical hollow-fiber modules. One pair is two modules each 12 cm long, for a total length of 24 cm; the other pair is two modules each 32 cm long, for a total length of 64 cm. Each module contains 96 microporous polypropylene hollow fibers of 240 μm internal diameter (Celgard X-20, Hoechst-Celanese, Charlotte, NC). The pores of the fibers are filled with cross-linked polyvinylalcohol gel. The procedure of gel coating is as follows. The fibers are treated with a solution of the surfactant Tween 60 and air-dried, leaving a layer of surfactant on the surface of hollow fibers' wall. After this pretreatment, the fibers are easily wet by an aqueous solution of 6% wt. PVA and 1% wt. divinylsulfone is pumped through the module. After the fibers are wet completely by the polymer solution, aqueous 20% wt. NaOH is pushed through the column to initiate the cross-linking. The cross-linking reaction is very fast: the fibers change color from white to yellow as the basic solution passes through the module. After the cross-linking is complete, the column is washed by water for about a half hour to remove all water-soluble impurities. Further details of this procedure are described elsewhere (Schisla, 1990; Ding, 1992).

The two gel-coated columns are connected in series as shown in Figure 1 (Ding and Cussler, 1991). The aqueous phase is pumped through the lumen of each fiber in the column with two metering pumps connected in parallel [FMI Lab Pump, Model #QG-6 with 1/8-in. (3.2-mm) pump head]. The octanol phase, prepared as above, is pumped into a shell side with another metering pump [FMI Lab Pump, Model# RRP with 1/8-in. (3.2-mm) pump head]. The feed soution containing racemic leucine is fed into the aqueous phase through a tee that connects the two hollow-fiber columns. The feed solution is delivered by a HPLC pump (Spectra-Physics IsoChrom Digital).

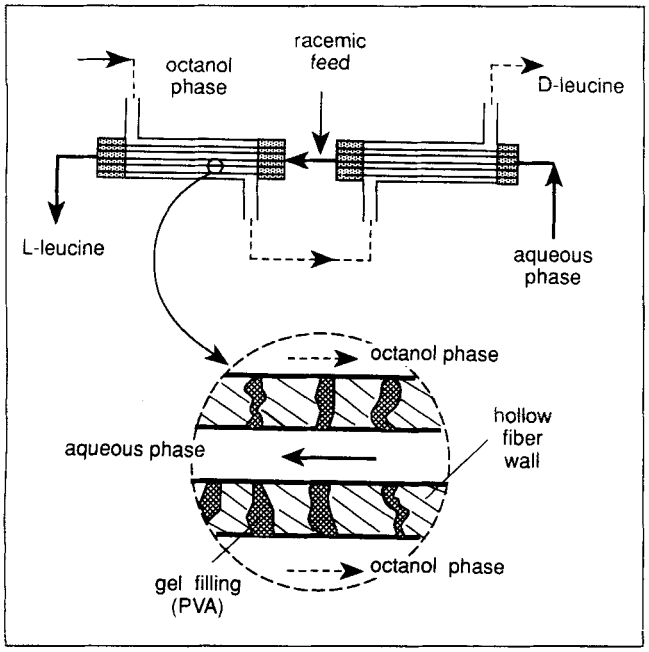

Figure 1. Fractional extraction with gel-coated hollow fibers.

The gel in the pores of the hollow-fiber membranes permits diffusion but prevents convection.

The procedure is as follows. The organic solution is pumped into the columns first. After the shell side of the columns is filled with the organic solution, the organic flow is stopped and the aqueous phase is pumped into lumen side of the column. After the lumen side is filled with aqueous solution, the organic flow is started again. When a stable flow is reached in both lumen and shell side, the feed flow is introduced. *D*- and *l*-leucine concentrations in the two products are measured by the chromatographic method described above. Other filling procedures sometimes entrained small amounts of water in the organic solution.

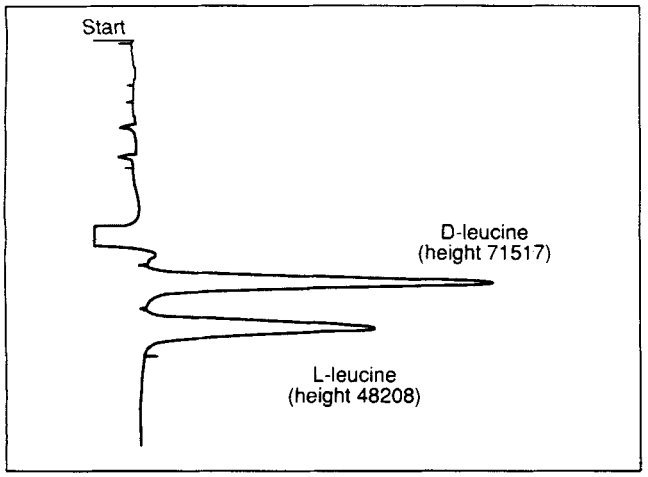

Figure 2. Separation of racemic leucine by reverse-phase HPLC.

D-leucine comes out earlier at 2.924 minutes and *l*-leucine comes out a little later at 3.485 minutes. The mobile-phase flow rate was 1.00 mL/min.

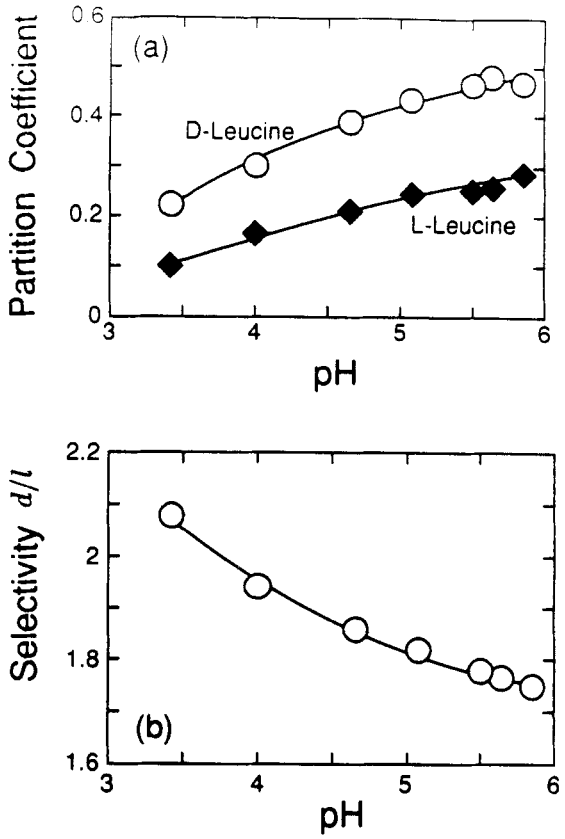

Figure 3. (a) Partition coefficients of leucine isomers increase with pH; (b) selectivity between leucine isomers in dodecyl-*l*-hydroxyproline solutions decrease with pH.

Results

This research shows how racemic leucine can be separated into *d*- and *l*-isomers by hollow-fiber extraction. Before we can demonstrate this separation, we first must prove that we can independently measure the amounts of individual isomers present in the solution. We must also measure the partition coefficients between the aqueous and organic phases, for these partition coefficients are an essential part of the theory of fractional extraction (Ding and Cussler, 1991).

Our measurements of the concentrations of individual isomers, made by high-performance liquid chromatography using the column described above, give a complete baseline separation as exemplified by the data in Figure 2. Experiments reported elsewhere (Ding, 1992) show that the peak height varies linearly with the isomer concentration when the concentration is lower than 1 mM. The feed mixture in Figure 2 is found to contain 51% *d*-leucine.

With this firm analytical basis, we then measure the partition coefficients for the two leucine isomers between the aqueous and organic phases. As detailed above, the aqueous phase contains cupric ion in an acetate buffer; the organic phase contains cupric ion and *n*-dodecyl-*l*-hydroxyproline in *n*-octanol. The partition coefficients of both isomers increase steadily with increasing pH, as shown in Figure 3a. However, the selectivity α, defined as the ratio of the partition coefficient of *d*-isomer to that of *l*-isomer, decreases with the increasing pH, as shown in Figure 3b. The difference in behavior as a

Table 1. Fractional Extraction for *dl*-Leucine from Two 32-cm Modules Containing 96 Fibers.*

Aqueous Flow mL/min	Aqueous Velocity cm/s	Extract Concentration, mM		Raffinate Concentration, mM		Fractions in Extract	
		$[d]_{org}$	$[l]_{org}$	$[d]_{org}$	$[l]_{org}$	$[d]$	$[l]$
0.14	0.05_4	0.043	<0.002	<0.002	0.169	1.000	0.00
0.21	0.08_1	0.058	<0.002	<0.002	0.221	1.00	0.00
0.32	0.12	0.056	<0.002	0.006	0.222	0.98	0.00
0.39	0.15	0.063	<0.002	0.002	0.236	1.00	0.00
0.63	0.24	0.058	0.002	0.004	0.222	0.98	0.03
0.81	0.31	0.058	0.002	0.016	0.233	0.94	0.03

* Experiment conditions: organic/aqueous flow = 4; feed/aqueous flow = 0.1; pH = 4.0; partition coefficients for *d*- and *l*-isomers = 0.331 and 0.160, respectively; and feed concentration = 4.9-mM leucine (51% *d*-leucine and 49% *l*-leucine).

function of pH reflects the presence of the two diasteromers and not just the two enantiomers. The *pKa*'s of the two diasteromers are different.

The values in Figure 3 both for the partition coefficients and for the selectivity are large for a chiral system, though small compared to most conventional extractions. Accordingly, we must use a large phase ratio of organic to aqueous phase, as we will need many transfer units to get a nearly complete separation. It can be difficult to operate a conventional extraction equipment under these conditions. It is relatively straightforward to operate hollow-fiber units under these conditions.

We now turn to the hollow-fiber extractions themselves, using the equipment shown in Figure 1. The samples from both the octanol phase and the aqueous phase are taken after the steady state is reached, typically reached after three to five column volumes of the aqueous-phase pass through the column. The analysis of the aqueous samples is the same as that for the partition experiments described above. The analysis of the octanol-phase samples is a little different. The octanol-phase sample is stripped with an equal volume of 0.2-M acetate buffer at pH 4.0. Then 0.5 mL of stripping solution is diluted with the 5.0-mL mobile phase and then analyzed. A set of typical results and operation conditions are shown in Table 1. More results for the hollow-fiber extractions are shown in Figure 4. In this figure, the fraction extracted into the organic phase is plotted vs. the velocity in the aqueous phase. The experiments were made at a constant phase ratio of 4.0, so that changes in the aqueous velocity imply corresponding changes in the organic-phase velocity. The pH was kept constant at 4.0, so the partition coefficients and the selectivity also remained constant.

The results in Table 1 and Figure 4 show that the organic phase is highly enriched in *d*-leucine, and considerably depleted in *l*-leucine. We periodically measured the concentrations in the aqueous phase, which is depleted in *d*-leucine but enriched in *l*-leucine. We found that the mass balance on all streams was within ±2% for each isomer. Accordingly, concentrations in the organic phase can easily be calculated from those in the aqueous phase.

The organic concentrations in Figure 4 can also be replotted vs. Graetz number on a single curve, as shown in Figure 5. In this case, the Graetz number is defined as $(d^2v/D\ell)$, where d is the hollow-fiber diameter, v is the aqueous velocity, D is the diffusion coefficient in water, and ℓ is the module length. Theories of hollow-fiber extraction (Prasad and Sikar, 1990; Ding and Cussler, 1991) suggest that the Graetz number is a key parameter. The theory (Eqs. 7-11, Ding and Cussler, 1991) predicts the dotted line shown in Figure 5, which appears to be in good agreement with the data. Reassuringly, the fraction extracted does begin to deviate from zero or one when the Graetz number is about one. However, the apparent agreement between theory and experiment may be fortuitous. We have varied ℓ and v, but not d and D. Casually, we have assumed that D refers to the aqueous phase, not the organic phase. While we believe that the data in Figure 5 are consistent with the theory of fractional extraction, they do not verify that theory.

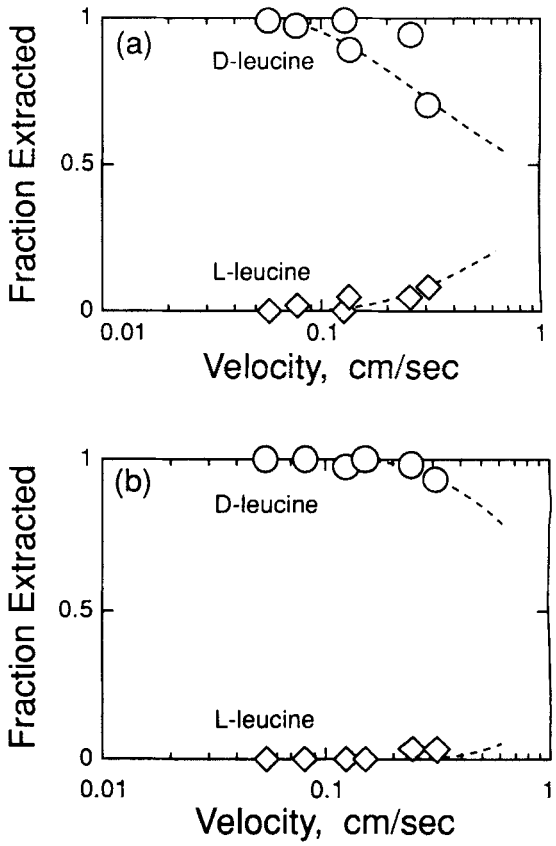

Figure 4. Hollow-fiber extractions of racemic leucine.

The data in (a) and (b) were obtained with twin columns which were 12 cm or 32 cm long, respectively. The organic velocity was always four times the aqueous velocity; the pH was always 4.0.

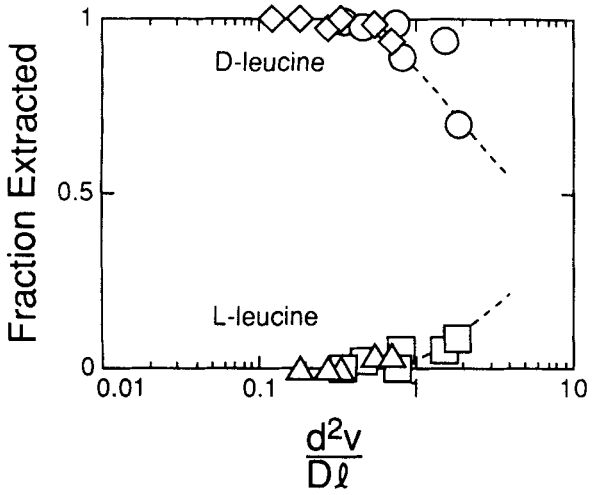

Figure 5. Fractional extraction vs. Graetz number.

All the leucine data collapse onto a pair of curves when plotted vs. Graetz number, as predicted theoretically. The data are taken from Figure 4; the lines are predictions (Ding and Cussler, 1991).

Discussion

In this section, we want to explore the implications of the extraction data given above for large-scale chiral separations. Before we begin this discussion, we need to state clearly what our data do not show. Our data are not a new system for separating amino acids like racemic leucine. We have copied the excellent system developed by Takeuchi et al. (1984, 1990). We have copied the compositions of the aqueous phase and the organic phase, and chemical details like cupric ion and *n*-octanol. Not surprisingly, we get similar partition coefficients and selectivities because we are not using new chemistry.

We are using a new geometry—based on hollow fibers—to achieve the leucine separation. This new geometry is very different from a conventional extraction column and from the high-efficiency rotating column. We want to compare the performance of these three extracting geometries. Our comparison with the rotating column is especially easy, because Takeuchi et al. (1990) used that geometry. Indeed, our desire to make this direct comparison is the chief reason that we imitated Takeuchi's extraction chemistry.

The three geometries are compared in detail in Table 2 for a separation yielding 99% pure *d*-leucine and 99% pure *l*-leucine. From the partition coefficients given above, we can easily calculate that this separation requires 27 transfer units (Ding and Cussler, 1991). The first column in Table 1 gives the type of extractor, and the second column gives the product of the mass-transfer coefficient k and the surface area per volume a. The value of ka for the hollow-fiber systems is shown in Figures 3-4 of our earlier work (1991); that for the rotating extractor is calculated from Takeuchi et al. (1990); and that for the conventional extractors is estimated from Treybal (1980). Note that ka is much larger for the hollow fibers than for the other two extractors. We believe that this ka is a consequence of the larger value of a, that is, of the high surface area per volume characteristics of hollow-fiber systems.

We considered trying to separate k and a, and listing these as two separate values in the table. Doing so is easy for the hollow fibers, because the area per module volume is just four divided by the fiber diameter and times the volume of fibers per volume of module. For the system used here, a equals 167 cm^2/cm^3. However, the area per volume of the rotating and conventional equipment is much more difficult to estimate. A rough guess for the rotating equipment is 2 cm^2/cm^3. However, no direct measurements of a for conventional equipment are available; correlations for conventional equipment give only the product ka.

Table 2. Comparison of Three Different Extractors*

Type Extractor	$ka \times 10^4$ s^{-1}	Residence Time [h]	Length [m]	Volume [cm^3]	Volumetric Capacity
Hollow Fiber	530	0.06	2×0.32	8	9
Rotating	7.0	5.4	2×1.0	630	0.12
Conventional	0.5	75	2×14	9,000	0.008

* Each extractor contains 27 transfer units and gives 99% isomerically pure *D*- and *L*-leucine.

The remaining columns in Table 2 explore the consequences of the values of ka and the need for 27 transfer units. Column 3 gives the residence time in the extractor: hollow fibers require 1% of the residence time of a rotating extractor and 0.1% of that of a conventional extractor. These smaller residence times mean that the equipment can be dramatically smaller for the same task, as suggested by the extractor lengths and volumes given in columns 4-5 of Table 2.

Still, comparing different pieces of equipment is difficult. There is simply no standard available. One comparison for these differential contactors might be in terms of the volumetric capacity:

$$[\text{volumetric capacity}] = \frac{\text{daily volume of feed}}{\text{total volume of equipment}}.$$

The volumetric capacities estimated in this way are listed in the last column of Table 2. On this basis, hollow fibers are 100 times more productive than the rotating extractor, and 1,000 times more effective than conventional units. A second, more sensible comparison would be volumetric capacity per equipment cost. We certainly expect that conventional equipment to be the cheapest, but we have no basis for estimating the cost of the hollow-fiber equipment; we only expect that it will be less expensive than the rotating extractor. More exact conclusions require further work.

Acknowledgment

This work was chiefly supported by the Hoechst-Celanese Corporation. Other support came from the National Science Foundation, the Petroleum Research Fund, and General Mills. E.L.C. held an Amundson Professorship during a sabbatical leave at MIT where he was graciously welcomed.

Literature Cited

Addadi, L., S. Weinstein, E. Gati, I. Weissbush, and M. Lahav, *J. Amer. Chem. Soc.*, **104**, 4610 (1982).
Ahuja, S., "Chiral Separations by Liquid Chromatography," Amer. Chem. Soc., Washington, DC (1991).
Dahuron, L., and E. L. Cussler, "Protein Extractions with Hollow Fibers," *AIChE J.*, **34**, 130 (1988).
Ding, H., "Hollow Fiber Liquid Chromatography and Fractional Extraction," PhD Thesis, Univ. of Minnesota (1992).
Ding, H., and E. L. Cussler, "Fractional Extraction with Hollow Fibers with Hydrogel-Filled Walls," *AIChE J.*, **37**, 855 (1991).
Pasteur, L., *Ann. Chim. Phys.*, **24**, 442 (1848).

Prasad, R., and K. K. Sirkar, "Hollow Fiber Solvent Extraction: Performances and Design," *J. Memb. Sci.*, **50**, 153 (1990).
Schisla, D. K., "Hollow Fiber Liquid Chromatography," PhD Thesis, Univ. of Minnesota (1990).
Takeuchi, T., R. Horikawa, and T. Tanimura, "Resolution of DL-Valine by Countercurrent Solvent Extraction with Continuous Sample Feeding," *Sep. Sci. Technol.*, **25**(7&8), 941 (1990).
Takeuchi, T., R. Horikawa, and T. Tanimura, "Enantioselective Solvent Extraction of Neutral DL-Amino Acids in Two-Phase Systems Containing N-n-Alkyl-L-Proline Derivatives and Copper (II) Ion," *Anal. Chem.*, **56**, 1152 (1984).
Treybal, R. E., *Mass Transfer Operations*, 3rd ed., McGraw-Hill, New York (1980).
Wernicke, R., "Separation of Underivatised Amino Acid Enantiomers by Means of a Chiral Solvent-Generated Phase," *J. Chromat. Sci.*, **23**, 39 (1985).

Manuscript received Mar. 19, 1992, and revision received July 21, 1992.