Anal. Chem. **1998,** *70,* 3717−3719

Correspondence

Bubble Fractionation of Enantiomers from Solution Using Molecularly Imprinted Polymers as Collectors

D. W. Armstrong,* J. M. Schneiderheinze, and Y.-S. Hwang

Department of Chemistry, University of Missouri−Rolla, Rolla, Missouri 65401

B. Sellergren

Department of Inorganic Chemistry and Analytical Chemistry, Johannes Gutenberg University−Mainz, J. J. Becherweg 24, D-56099 Mainz, Germany

Adsorptive bubble separation methods have been used to enrich components from both heterogeneous and homogeneous solutions. These methods are particularly effective for processing large solution volumes at low cost. Previous work demonstrated that chiral, surface-active collectors could be used to enrich enantiomers from homogeneous solution in a foam fractionation process. In a significant extension of this work, the use of highly selective molecularly imprinted polymers (MIPs) and heterogeneous solutions for the bubble flotation of enantiomers was evaluated. The high selectivity and ease of recycling of the MIP make this a potentially powerful approach for process-scale separations from large-volume bulk solutions. New MIPs were produced with low swelling properties which allowed them to retain enantioselectivity after numerous recyclings.

Molecularly imprinted polymers[1−5] have been used to prepare chiral stationary phases for chromatography,[6−12] as substitutes for antibodies in immunoassay protocols,[13,14] as biomimetic sensors,[15] and as selective media for solid-phase extraction.[16−21] Imprinted polymers can have very high selectivities for specific molecules, even enantiomers.[6−12] This high selectivity can be counterproductive for some analytical applications, where a lower but adequate selectivity for a broad range of compounds is desired. However, the strength of molecularly imprinted materials is that their selectivity can be tailored for specific applications by using a variety of appropriate molecular templates. The ability to engineer very high selectivity for a specific molecule could be useful for preparative- or process-scale separations. There is an inverse relationship between selectivity and the number of theoretical plates needed in countercurrent separation procedures. If selectivity is sufficiently high, complete separation can be achieved in a one-step process (1 theoretical plate).

Adsorptive bubble separation processes can be very useful for isolating large amounts of material at very low costs. Foam flotation is an important technique for concentrating sulfide ores.[22] Metal ions and chelates in aqueous solution can be enriched using foam fractionation methods.[23−27] In one unusual application, the foam fractionation of enantiomers was accomplished from aqueous

(1) Mosbach, K. *Trends Biochem. Sci.* **1994**, *19*, 9−14.
(2) Wulff, G. *Trends Biotechnol.* **1993**, *11*, 85−87.
(3) Shea, K. J. *Trends Polym. Sci.* **1994**, *2*, 166−173.
(4) Sellergren, B. *TrAC, Trends Anal. Chem.* **1997**, *16*, 310−320.
(5) Mayes, A. G.; Mosbach, K. *TrAc, Trends Anal. Chem.* **1997**, *16*, 321−332.
(6) Sellergren, B.; Lepistö, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, *110*, 5853−5860.
(7) Fischer, L.; Muller, R.; Ekberg, B.; Mosbach, K. *J. Am. Chem. Soc.* **1991**, *113*, 9358−9360.
(8) Andersson, L. I.; Mosbach, K. *J. Chromatogr.* **1990**, *516*, 313−322.
(9) Kempe, M.; Mosbach, K. *J. Chromatogr. A* **1995**, *694*, 3−13.
(10) Wulff, G.; Minarik, M. *J. Liq. Chromatogr.* **1991**, *13*, 2987−3000.
(11) Schweitz, L.; Andersson, L. I.; Nilsson, S. *Anal. Chem.* **1997**, *69*, 1179−1183.
(12) Vidysankar, S.; Ru, M.; Arnold, R. H. *J. Chromatogr. A* **1997**, *755*, 51−63.
(13) Vlatakis, G.; Andersson, L. I.; Muller, R.; Mosbach, K. *Nature* **1993**, *361*, 645−647.
(14) Andersson, L. I.; Muller, R.; Vlatakis, G.; Mosbach, K. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4788−4792.
(15) Kriz, D.; Ramstrom, O.; Svensson, A.; Mosbach, K. *Anal. Chem.* **1995**, *67*, 2142−2144.
(16) Sellergren, B. *Anal. Chem.* **1994**, *66*, 1578−1582.
(17) Muldoon, M. T.; Stanker, L. H. *Anal. Chem.* **1997**, *69*, 803−808.
(18) Matsui, J.; Okada, M.; Tsuruoka, M.; Takeuchi, T. *Anal. Commun.* **1997**, *34*, 85−87.
(19) Andersson, L. I.; Paprica, A.; Arvidsson, T. *Chromatographia* **1997**, *46*, 57−62.
(20) Martin, P.; Wilson, I. D.; Morgan, D. E.; Jones, G. F.; Jones, K. *Anal. Commun.* **1997**, *34*, 45−47.
(21) Rashid, B. A.; Briggs, R. J.; Hay, J. N.; Stevenson, D. *Anal. Commun.* **1997**, *34*, 303−305.
(22) Rossberg, K. S. E., Ed. *Flotation of Sulfide Minerals*; Elsevier: Amsterdam, 1985.
(23) Rusanov, A. I.; Levicer, S. A.; Zarov, V. T. *Povercknostnage Radelene Vercestv*; Ezd. Chimija: Leningrad, 1981.
(24) Golman, A. M. *Ionnaja Flotacija*; Nedra: Moscow, 1982.
(25) Grieves, R. B. In *Treatise on Analytical Chemistry*, 2nd ed.; Elving, P. F., Ed.; Wiley-Interscience: New York, 1982; Vol. 5.
(26) Wilson, D. J.; Clarke, A. N. *Topics in Foam Flotation*; Dekker: New York, 1983.
(27) Walkowiak, W. *Sep. Sci. Technol.* **1991**, *26*, 559−568.

S0003-2700(98)00154-1 CCC: $15.00
Published on Web 07/17/1998

solutions using dissolved chiral collectors (i.e., foaming agents).[28] For such a separation to be practical on a large scale, a high enrichment factor is needed, and the chiral collector should be easily recyclable. Dissolved collectors are generally more difficult to isolate than solid ones, which can be filtered, centrifuged, or allowed to settle under the influence of gravity. Also, even though the dissolved chiral collectors in the aforementioned report[28] produced enantiomeric enrichments using a countercurrent foam column, still greater enantioselectivity would be desirable.

The use of a solid, chiral imprinted polymer as a collector for the bubble flotation of enantiomers has not been considered, to our knowledge. However, the ability to obtain molecularly imprinted particles with high enantioselectivity, coupled with the fact that solids can be easily isolated and recycled, could be very advantageous for a bubble separation process. In this communication, the feasibility of fractionating enantiomers using molecularly imprinted polymeric collectors and bubble flotation is examined.

EXPERIMENTAL SECTION

Materials. The polymer was prepared using a previously published procedure with a few modifications.[29] The composition of the polymer was approximately 17 mol % methacrylic acid and 83 mol % ethyleneglycol dimethacrylate. Both the molecularly imprinted polymer (MIP) and an unimprinted "blank" were made. The unimprinted polymers were used to test for and identify nonenantioselective adsorption. To compare MIPs with different porosities and swelling, methylene/chloride and acetonitrile were used as porogens as follows. L-Phenylalanine anilide (0.96 g, 4 mmol), methacrylic acid (1.36 mL, 16 mmol), ethyleneglycol dimethacrylate (15.2 mL, 80 mmol), and azobis(isobutyronitrile) (160 mg, 1.0 mmol) were dissolved in 22 mL of acetonitrile or methylene chloride. The solutions were sparged with nitrogen gas for about 5 min and then transferred to six thick-walled glass polymerization tubes, again sparged with nitrogen gas and sealed with Parafilm. The tubes were then immersed in a water bath maintained at 15 °C and allowed to equilibrate for 10 min. The tubes were irradiated by a high-pressure Hg lamp for 24 h, rotating the tube periodically to ensure even polymerization. Following polymerization, the polymer monolith was ground in a wet state by means of a mechanical ball mill. Size fractionation of the ground MIP was done by mechanical seiving under water. The MIP consisted of irregular particles ranging from approximately 0.5 to 20 μm in size, as measured using optical microscopy. The foaming device was similar to that used by Armstrong et al., with some modifications.[28] The top of the foaming column was removed by heating with a Bunsen burner, leaving a cylindrical column approximately 25 cm in height. A ledge, approximately 1 cm wide, was then formed at the top of this column by heating and shaping the glass. As air was introduced through a frit at the bottom sample chamber, the bubbles carried the MIP to the top of the column, where it was concentrated. The concentrated MIP was easily transferred manually (with a spatula) to the glass ledge. The interior of the glass column was filled with glass beads 0.7 cm in diameter, which served to decrease the average bubble size and produce a drier foam. The column contained indentations at the top and bottom to prevent the removal of the glass beads.

Chromatography. High-performance liquid chromatography (HPLC) grade methanol, acetonitrile, triethylamine, and acetic acid were purchased from Fisher Scientific Co. (St. Louis, MO). All samples were analyzed using a Cyclobond I 2000 SN HPLC column (Advanced Separation Technologies, Whippany, NJ) in the polar organic mode. The mobile phase composition was 99:1:0.2:0.6 (acetonitrile/methanol/triethylamine/acetic acid) and was delivered at 1 mL/min using a Shimadzu LC-6A pump (Kyoto, Japan). Detection was accomplished using a Shimadzu SPD-2AM UV−vis detector at a wavelength of 254 nm. Detector output was recorded using a Shimadzu CR-601 chromatopac integrator.

Enrichment and Extraction Procedures. Prior to each use, the polymer was washed with 300 mL (2 × 150 mL) of 5% acetic acid in acetonitrile. A known mass of the polymer was accurately measured and added to a 20-mL sample vial. To this vial was added 10 mL of 30:70 (acetonitrile/1% triethylammonium acetate buffer adjusted to a pH of 4.1). A mixture of approximately equal amounts of L- and D-phenylalanine anilide was prepared and dissolved in a known amount of acetonitrile. A known volume of this mixture was transferred either to the sample compartment at the bottom of the bubble separation device or to a separate vial containing a suspension of the MIP. The heterogeneous mixture of MIP and analyte was stirred for 30 min. Subsequently, the MIP was isolated by bubble flotation or filtration. The isolated material was rinsed with successive 5-mL portions of 30:70, acetonitrile/1% triethylammonium acetate buffer (pH = 4.1) to ensure removal of any surface-adsorbed analyte. The polymer was then rinsed with 5−10 mL of 5% acetic acid in acetonitrile to extract any sample from the selective pores of the MIP. The solution was then suction filtered using an aspirator. The filtered solution was transferred to a clean 20-mL sample vial and was then concentrated by evaporating the solvent using an aspirator. After concentration, the recovered sample was analyzed by HPLC. The polymer was then washed with 300 mL (2 × 150 mL) of 5% acetic acid in acetonitrile to ensure that any remaining sample was removed from the binding sites and that the acidic binding sites of the MIP were protonated before the next use.

Loading Studies. Experiments were performed to determine if the amount of racemate applied to the polymer influenced the enrichment of the recovered sample. In all experiments, approximately 0.71 g of the imprinted polymer was used. Seven experiments were performed using between 1 and 100 μg of racemic phenylalanine anilide solution following the aforementioned procedure.

RESULTS AND DISCUSSION

There are two basic requirements for the successful bubble fractionation of enantiomers using molecularly imprinted polymers. First, solid MIP particles must adhere to bubbles and be efficiently transported to the surface of an appropriate solution. Second, the enantioselectivity of the MIP must be appreciable, since large numbers of countercurrent equilibration steps (e.g., theoretical plates) are not available in this technique as they are in HPLC and CE, for example. It was determined empirically (see Experimental Section) that MIP particles in the range of ∼0.1−20 μm in diameter adhered to air bubbles and were effectively transported to the top of the column in both aqueous solutions

(28) Armstrong, D. W.; Zhou, E. Y.; Chen, S.; Le, K.; Tang, Y. *Anal. Chem.* **1994**, *66*, 4278−4282.
(29) Sellergren, B.; Shea, K. J. *J. Chromatogr.* **1993**, *635*, 31−49.

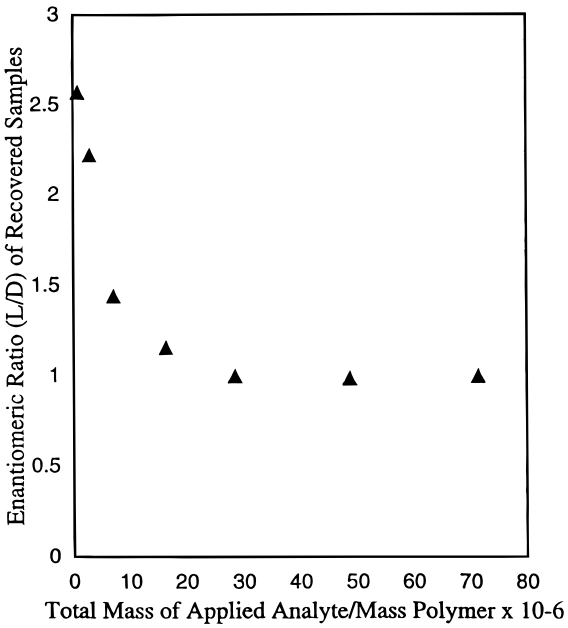

Figure 1. Plot of the enantiomeric ratio of L-phenylalanine anilide/D-phenylalanine anilide vs the ratio of mass racemate/mass polymer.

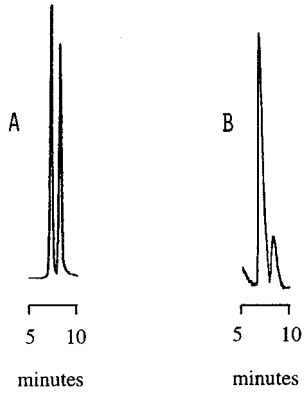

Figure 2. (A) Enantiomeric separation of DL-phenylalanine anilide racemic standard and (B) the enantiomeric composition of DL-phenylalanine anilide recovered from the molecularly imprinted polymer. The starting solution for (B) contained approximately 1 μg of analyte/g of molecularly imprinted polymer. Both separations were performed on a Cyclobond I 2000 SN HPLC column using a mobile phase of 99:1:0.2:0.6 (acetonitrile/methanol/triethylamine/acetic acid) with a flow rate of 1 mL/min at ambient temperature. Detection was accomplished at 254 nm.

and hydroorganic solutions containing as much as 30% acetonitrile (by volume). Particles >40 μm in diameter were not effectively carried and concentrated at the solution−air interface by a bubble/foam process.

The enantioselective adsorption of phenylalanine anilide by the MIP particles was very sensitive to analyte loading. This was noted previously in HPLC studies.[29,30] Figure 1 shows the effect of analyte loading on the enantiomeric ratio of phenylalanine anilide recovered from the MIP prepared using acetonitrile as the porogen. Note that when the amount of phenylalanine anilide in solution exceeds ~25 μg/g of MIP, the enantiomeric enrichment is not as favorable. At concentrations ≥50 μg/g of MIP, the isolated product appears to be racemic. When these experiments were repeated with the same polymeric collector which had not been molecularly imprinted, no enantiomeric enrichment was observed at any analyte concentration (even though nonselective binding was observed). Clearly, there are at least two binding sites for the phenylalanine anilide on the MIP. The adsorption isotherm of D- and L-phenylalanine anilide can be fitted with bi-Langmuir isotherm models consisting of one small class of sites with high binding energy and high enantioselectivity and another larger class of weaker sites with low or no enantioselectivity.[30] Since, the number of imprinted, stereoselective binding sites is limited, high concentrations of analyte can result in nonselective adsorption. Figure 2 shows the chromatographic results indicating enantioselective binding of phenylalanine anilide on the MIP after a single equilibration step under optimized conditions. Nearly 90% of the product was L-phenylalanine anilide.

MIPs are known to swell and contract when exposed to different solvents.[6] The degree to which they swell and shrink depends on their chemical makeup, degree of cross-linking, and other factors inherent in the manufacturing process.[1−5,29] The enantiomeric enrichment of L- to D-phenylalanine anilide by the MIP (~1 μg of analyte/g of MIP) decreased from 72/28 to 63/37 by the fourth solvent cycle. However, this MIP, prepared using methylene chloride as porogen, is known to undergo significant swelling in acetonitrile. Apparently, the stereoselective molecular imprint can gradually be lost if the swelling and shrinking are excessive or if the MIP undergoes enough "swell/shrink cycles". Alternatively, the loss in selectivity may be caused by incomplete extraction between runs or insufficient equilibration. To examine this process further, we prepared a "low-swell" MIP using acetonitrile as the porogen (see Experimental Section). The enantioselectivity of the "low-swell" MIP was completely analogous to that of the original material. However, after recycling the "low-swell" MIP 12 times, no significant decrease in the enantioselectivity was observed. Clearly, relatively simple modifications in the synthesis of a MIP can affect its ruggedness as well as its selectivity and loadability.

This work indicates that molecularly imprinted polymers can be used to selectively concentrate and purify dissolved solutes (including enantiomers) using economical adsorptive bubble procedures. Adsorptive bubble techniques are particularly well suited to continuous processes that involve large volumes of liquid. Hence, in addition to laboratory chemical separations, they (coupled with appropriate MIPs) also could be useful for selective treating and/or analysis of large amounts of wastewater and other bulk solutions. By modifying the synthesis of a MIP, one can control its ruggedness, selectivity, and loadability.

ACKNOWLEDGMENT

Support by the National Institute of Health (NIH R01 GM53825-01) and The Seoul Metropolitan Government Institute of Health and Environment, Seoul, South Korea, is gratefully acknowledged.

Received for review February 10, 1998. Accepted June 4, 1998.

AC980154L

(30) Sajonz, P.; Kele, M.; Zhong, G.; Sellergren, B.; Guichon, G. *J. Chromatogr.* **1998**, *810*, 1−17.