ORGANIC LETTERS

2002
Vol. 4, No. 17
2937−2940

Measurement of Enantiomeric Excess Using Molecularly Imprinted Polymers

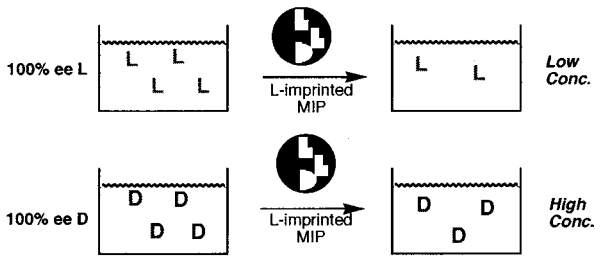
Yizhao Chen and Ken D. Shimizu*

University of South Carolina, Department of Chemistry and Biochemistry, Columbia, South Carolina 29208

shimizu@mail.chem.sc.edu

Received June 10, 2002

ABSTRACT



A new method is presented for the measurement of enantiomeric excess (ee) utilizing molecularly imprinted polymers (MIPs). The method is demonstrated to be accurate and rapid, as the ee values can be calculated from straightforward concentration measurements. The MIP-based assay can also be adapted to measure the ee of samples of differing initial concentrations.

In the past few years, there has been a resurgence in the search for new methods for rapid measurement of enantiomeric excess (ee)[1] for use in combinatorial and high-throughput asymmetric catalyst discovery and optimization.[2,3] Current methods for measuring ee that utilize chiral HPLC and GC are not well suited for high throughput assays because they are serial methods that analyze one sample at a time. To address this issue, a number strategies have recently been developed that allow high-throughput ee analysis using "psuedo-enantiomers",[4] CD-spectroscopy,[5] kinetic resolution using enzymes or enantioselective reagents,[6] parallel screening of individual enantiomers,[7] and enantioselective sensors.[8] We present here a new method for rapidly testing samples for enantiomeric excess using binding assays based on synthetic molecularly imprinted polymers (MIPs). The methodology has the advantages of being quick and inexpensive and having the potential for being easily extended to a wide range of chiral molecules.

MIPs are highly cross-linked polymers that can be tailored with selectivity for a desired chiral guest.[9] When a chirally pure template molecule is used, the generated binding sites are enantioselective. In many respects, MIPs compare

(1) (a) Reetz, M. T. *Angew. Chem., Int. Ed.* **2001**, *40*, 284−310. (b) Wahler, D.; Reymond, J. L. *Curr. Opin. Biotechnol.* **2001**, *12*, 535−544.

(2) (a) Gilbertson, S. R. *Prog. Inorg. Chem.* **2001**, *50*, 433−471. (b) Dahmen, S.; Brase, S. *Synthesis* **2001**, 1431−1449. (c) Shimizu, K. D.; Snapper, M. L.; Hoveyda, A. H. *Chem. Eur. J.* **1998**, *4*, 1885−1889.

(3) (a) Liu, G. C.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 7712−7713. (b) Gilbertson, S. R.; Wang, X. F. *Tetrahedron Lett.* **1996**, *37*, 6475−6478. (c) Cole, B. M.; Shimizu, K. D.; Krueger, C. A.; Harrity, J. P. A.; Snapper, M. L.; Hoveyda, A. H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1668−1671. (d) Reetz, M. T.; Zonta, A.; Schimossek, K.; Liebeton, K.; Jaeger, K. E. *Angew. Chem., Int. Ed.* **1997**, *36*, 2830−2832. (e) Porte, A. M.; Reibenspies, J.; Burgess, K. *J. Am. Chem. Soc.* **1998**, *120*, 9180−9187. (f) Gennari, C.; Ceccarelli, S.; Piarulli, U.; Montalbetti, C.; Jackson, R. F. W. *J. Org. Chem.* **1998**, *63*, 5312−5313. (g) Francis, M. B.; Jacobsen, E. N. *Angew. Chem., Int. Ed.* **1999**, *38*, 937−941.

(4) (a) Reetz, M. T.; Becker, M. H.; Klein, H. W.; Stockigt, D. *Angew. Chem., Int. Ed.* **1999**, *38*, 1758−1761. (b) Janes, L. E.; Kazlauskas, R. J. *J. Org. Chem.* **1997**, *62*, 4560−4561.

(5) (a) Reetz, M. T.; Kuhling, K. M.; Hinrichs, H.; Deege, A. *Chirality* **2000**, *12*, 479−482. (b) Angelaud, R.; Matsumoto, Y.; Korenaga, T.; Kudo, K.; Senda, M.; Mikami, K. *Chirality* **2000**, *12*, 544−547.

(6) (a) Korbel, G. A.; Lalic, G.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 361−362. (b) Guo, J. H.; Wu, J. Y.; Siuzdak, G.; Finn, M. G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1755−1758.

(7) (a) Reetz, M. T.; Becker, M. H.; Kuhling, K. M.; Holzwarth, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2647−2650. (b) Jarvo, E. R.; Evans, C. A.; Copeland, G. T.; Miller, S. J. *J. Org. Chem.* **2001**, *66*, 5522−5527.

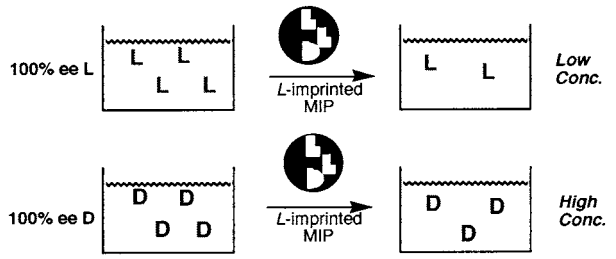
(8) (a) Kubo, Y.; Maeda, S.; Tokita, S.; Kubo, M. *Nature* **1996**, *382*, 522−524. (b) Pugh, V. J.; Hu, Q. S.; Pu, L. *Angew. Chem., Int. Ed.* **2000**, *39*, 3638−3641. (c) Reetz, M. T.; Sostmann, S. *Tetrahedron* **2001**, *57*, 2515−2520. (d) van Delden, R. A.; Feringa, B. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3198−3200. (e) Yan, Y.; Myrick, M. L. *Anal. Chem.* **1999**, *71*, 1958−1962.

10.1021/ol026336q CCC: $22.00
Published on Web 07/24/2002

favorably with other artificial enantioselective receptors such as engineered antibodies or synthetic molecular receptors. They have excellent thermal and chemical stability and are easily tailored with selectivity for a particular analyte. Most importantly, MIPs are easily prepared. Commonly in our laboratories, we prepare MIPs on a 1−20 g scale from commercially available starting materials in 2 days. For these reasons, MIPs have been utilized in a wide array of enantioselective applications including chiral chromatography, sensing, and catalysis.[9]

The MIP ee assay is based on the principle that equilibration of solutions of differing ee with an enantioselective MIP will result in solutions of differing concentrations. For example in Scheme 1, an L-enantiomer solution is efficiently

Scheme 1

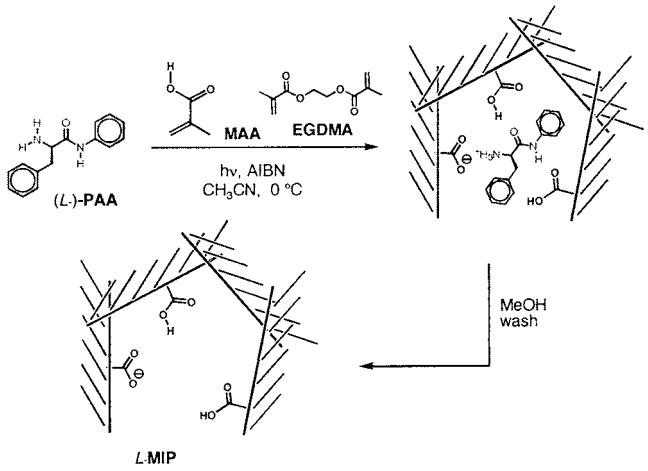


depleted by an L-selective MIP, resulting in a low concentration solution. A D-enantiomer solution, on the other hand, is less efficiently depleted, resulting in a higher concentration solution. These two examples represent the extremes and solutions of lesser ee should have intermediate concentrations. These differences in concentration should be easily measured and then can be correlated to the initial ee of the solution by comparison to a calibration curve. The assay is similar to ee assays based on proteins[8e] and antibodies,[10] except that the proteins have been replaced with more assessable and robust MIPs.

The system chosen to demonstrate the utility of MIPs in measuring ee's was a well-studied enantioselective polymer (L-MIP) imprinted with L-phenylalanine anilide (L-PAA).[11] The polymer was synthesized from the copolymerization of methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) in the presence of L-PAA in acetonitrile (Scheme 2). The resulting highly cross-linked polymer monolith was ground to a powder and washed by Soxhlet extraction with methanol to remove the template molecule.

To test whether there was a quantitative correlation between ee and concentration, 1.2 mM acetonitrile solutions of L-PAA and D-PAA of varying ee's were equilibrated with

Scheme 2



L-MIP. Concentration changes of up to 30% were observed (Figure 1), which were easily quantifiable by UV spectroscopy (260 nm). A binomial fit for the calibration curve gave an $R^2 = 0.9916$.[12]

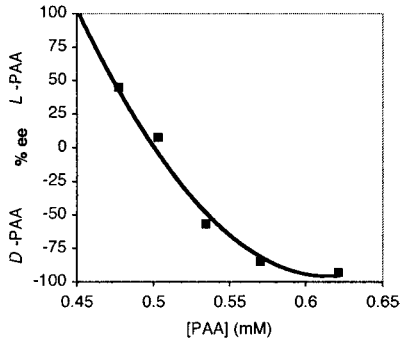


Figure 1. Calibration curve showing the concentrations of different ee PAA solutions (1.2 mM, CH_3CN) that were equilibrated with L-MIP as measured by UV (260 nm) spectroscopy.

Using the calibration curve, the accuracy of the methodology was tested using samples of known ee. Again the PAA samples (1.2 mM) in acetonitrile were equilibrated with L-MIP and the concentration of the resulting solution measured by UV. An excellent correlation was found between the actual ee as independently measured by chiral HPLC and the ee measured by the MIP-based assay (Figure 2). Individual measurements were found to have a respectable ±5% ee standard error.

The use of MIPs in the ee assay has a number of advantages: (1) The procedure is experimentally straightforward and does not require chromatography, chemical reactions, or difficult time-dependent concentration measurements. Simply adding a constant weight of polymer to a

(9) (a) Sellergren, B. *Molecularly Imprinted Polymers. Man Made Mimics of Antibodies and Their Applications in Analytical Chemistry*; Elsevier: Amsterdam, 2001. (b) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812−1832.

(10) Taran, F.; Gauchet, C.; Mohar, B.; Meunier, S.; Valleix, A.; Renard, P. Y.; Creminon, C.; Grassi, J.; Wagner, A.; Mioskowski, C. *Angew. Chem., Int. Ed.* **2002**, *41*, 124−127.

(11) Sellergren, B.; Lepistö, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, *110*, 5853−5860.

(12) There is no physical reason for the choice of this fitting equation. It was chosen because it provided the best empirical fit.

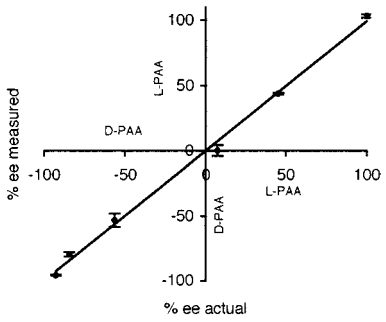


Figure 2. Correlation plot of actual and measured ee using the MIP ee assay.

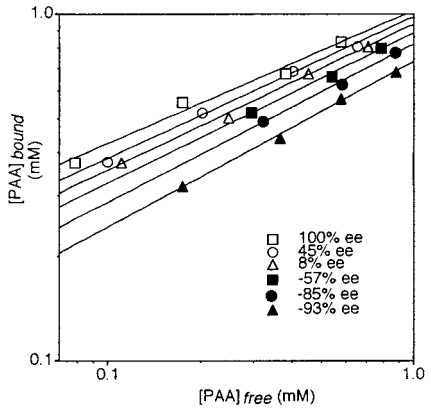


Figure 3. Calibrations curves of free and bound concentration of PAA solutions of varying ee tested over a range of concentrations after equilibration with L-PAA, plotted in log−log format.

constant volume of unknown solution, followed by a concentration measurement, yields the ee. (2) The necessary enantioselective MIPs can be generated inexpensively and in multigram scale from commercially available monomers.

Interestingly, the analysis was more sensitive toward the "mismatched" D-enantiomer than the imprinted L-enantiomer. This is apparent from the diminishing slope of the calibration curve (Figure 1) as it approaches 100% D-PAA. In this region, larger changes in concentration correlate to smaller differences in ee than for the L-PAA solutions. A possible explanation for this higher sensitivity for the mismatched D-enantiomer is that the small amounts of L-PAA in the high ee solutions of D-PAA (>80%) are almost quantitatively adsorbed by the L-MIP, leading to large changes in concentration. In contrast in the L-PAA solutions, the higher concentrations of L-PAA have already saturated the majority of L-PAA sites and therefore a smaller fraction of the L-PAA is bound.

A limitation of the above procedure and also of other high-throughput ee assays is that they require the samples to have the same initial concentrations. This restricts the utility of the methodology. Therefore, we sought to expand the MIP ee assay to accommodate differences in initial sample concentrations.

A series of calibration curves were made with six different ee solutions over a range of concentrations (0.5−2.0 mM). These were plotted as binding isotherms ($[PAA]_{bound}$ versus $[PAA]_{free}$, Figure 3). The $[PAA]_{free}$ was measured directly, and $[PAA]_{bound}$ was calculated as the difference between the measured values of $[PAA]_{initial}$ and $[PAA]_{free}$. In this format, the binding behavior of MIPs can be accurately modeled by the Freundlich isotherm (eq 1), which was confirmed by their

$$[PAA]_{bound} = a[PAA]_{free}^{\ m} \quad (1)$$

linear relationships when plotted in log−log format.[13]

To find the relationship between the initial ee of the solutions and corresponding $[PAA]_{free}$ and $[PAA]_{initial}$ concentrations, the variance in the Freundlich fitting parameters (a and m) with respect to ee was determined by individually plotting a and m versus ee.[14] These plots were empirically fit to biexponential and trinomial functions, respectively (eqs 2 and 3). These empirical expressions for a and m were then

$$a = -0.323\, e^{0.139(ee+100)} + 0.842\, e^{0.000972(ee+100)} \quad (2)$$

$$m = -4.24 \times 10^{-8}(ee + 100)^3 + 1.45 \times 10^{-5}(ee + 100)^2 - 0.00171(ee + 100) + 0.485 \quad (3)$$

inserted into eq 1 to yield an equation that allowed the calculation of ee from the initial ($[PAA]_{initial}$) and final ($[PAA]_{free}$) free analyte concentrations. This composite equation could not be solved for ee directly but instead was solved iteratively using the Solver function in Excel.

To test the accuracy of the methodology, 24 solutions of PAA of varying ee (100% ee L-PAA to 93% ee D-PAA) and varying concentrations (0.5−1.5 mM) were measured. Two concentration measurements were taken for each sample: an initial ($[PAA]_{initial}$) and a final ($[PAA]_{free}$) concentration using UV (260 nm). From these values, ee's were calculated from the combination of eqs 1−3. The accuracy of the ee measurements was verified by plotting the calculated ee's against the actual ee's as measured by chiral HPLC (Figure 4). Again, an excellent correlation was observed as seen by the straight line fit with a slope of 1.01. The error in the analysis increased to ±13% standard error. The error, however, is not weighted equally, and as was seen earlier, the analysis is more accurate for the mismatched enantiomer D-PAA (±5%, SE) than for the matched L-PAA (±17%, SE).

In conclusion, we have demonstrated that MIP binding assays are a viable method for rapidly measuring enantioselectivity with a relatively high degree of accuracy. Certainly, this methodology does have some limitations. Like other ee assays, the MIP-based assay is fairly specific for a particular

(13) (a) Chen, Y. B.; Kele, M.; Sajonz, P.; Sellergren, B.; Guiochon, G. *Anal. Chem.* **1999**, *71*, 928−938. (b) Umpleby, R. J.; Baxter, S. C.; Bode, M.; Berch, J. K.; Shah, R. N.; Shimizu, K. D. *Anal. Chim. Acta* **2001**, *435*, 35−42.

(14) Calculations are shown in Supporting Information.

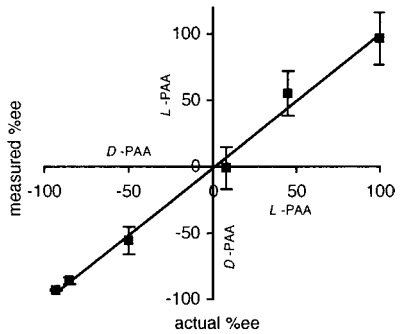


Figure 4. Correlation plot of actual versus calculated ee in the MIP assay in which initial concentrations of PAA were varied.

analyte (L-PAA). However, the MIP-based assay may have the advantage of being more easily tailored to new analytes than other molecular receptor based assays, such as synthetic enantioselective hosts or engineered antibodies. MIPs can be readily generated in a single step for a wide range of chiral analytes including steroids, carbohydrates, and amino acids.[9] Second, the MIP-based assay measured the concentrations of analyte using UV spectroscopy. However, UV activity of the analyte is not an intrinsic requirement for the assay. Any method of measuring concentration could be applied, including fluorescence, NMR, GC, HPLC, or scintillation. UV or fluorescence spectroscopy, however, are particularly attractive because of the potential of being easily adapted to analysis in a microtiter tray reader for applications in a high-throughput screening assay. We are currently in the process of investigating this possibility in which the solutions are equilibrated with MIPs in test tubes and then the resulting solutions are pipetted into microtiter trays for high-throughput ee screening.

Acknowledgment. We thank the NIH (R01-GM62593) for financial support.

Supporting Information Available: Experimental procedure for the synthesis of PAA and L-MIP and the high-throughput screening methodology and analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

OL026336Q