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Enantioselective Liquid–Solid Extraction (ELSE)—An Unexplored, Fast, and Precise Analytical Method

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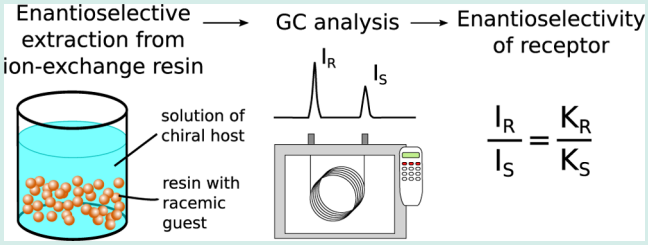
Supporting Information

ABSTRACT: A novel method of evaluating the enantioselectivity of chiral receptors is investigated. It involves extraction of an ionic guest in racemic form from an ion-exchange resin to the organic solvent, where it is bound by a chiral receptor. The enantioselectivity of the examined receptor is determined simply by measuring the enantiomeric excess of the extracted guest. We show that the concept is viable for neutral receptors binding chiral organic anions extracted into acetonitile. This method was determined to be more accurate and far less time-consuming than the classical titrations. Multiple racemic guests can be applied to a resin in a single experiment, giving the method a very high throughput.



KEYWORDS: *enantioselective extraction, chiral recognition, host–guest interactions, anion binding, high throughput*

Chiral recognition is a very subtle phenomenon, based on stereochemically dependent weak interactions. It is ubiquitous and substantial in biological systems, and one of the ways it manifests itself is in the differing actions of enantiomeric drug molecules. Efforts to investigate novel artificial enantiodiscriminating receptors are driven by two main factors. The first is the applicability of such compounds, mainly in the field of analytical chemistry, as chiral stationary phase modifiers (CSP)[1,2] and optical or electrochemical sensors.[3−8] The second is the pursuit of a better understanding of the chiral recognition phenomena, by performing receptor structure–enantioselectivity correlations.

Studies on artificial receptors consist of three stages: (a) design, (b) synthesis, and (c) evaluation of enantioselectivity. The last step is nowadays the most time-consuming and the least reliable.[9] Usually the ratio of association constants (α, eq 4) of the complexes of chiral host (H*) with enantiomers of guest (G_S and G_R) is used as a measure of chiral recognition.

$$H^* + G_S \rightleftharpoons H^*G_S \quad (1)$$

$$H^* + G_R \rightleftharpoons H^*G_R \quad (2)$$

$$K_S = \frac{[H^*G_S]}{[H^*]\cdot[G_S]} \qquad K_R = \frac{[H^*G_R]}{[H^*]\cdot[G_R]} \quad (3)$$

$$\alpha = \frac{K_S}{K_R} \quad (4)$$

In the classical approach both K values are determined in separate titration experiments. The typical uncertainty of such measurements is 5−15%, which results in the uncertainty of the ratio of these values (α) being no less than 7%. Since the optimal enantioselectivity for CSP in analytical HPLC or GC is around 1.1,[1] the classical method, with its high uncertainty, possess restricted applicability in this range of low selectivities. The high level of error makes the selectivity–structure correlations difficult, as some subtle effects may be lost. Titration experiments are truly time-consuming procedures: typically 15−30 min for UV–vis (fluorescence), 40−60 min for NMR, and 2−6 h for calorimetric titration.

Seeking to overcome the aforementioned problems, various methods have been developed, such as competitive NMR titration,[10−17] competitive UV–vis titrations,[18] and enantioselective liquid–liquid extraction (ELLE).[19−22] The competitive titration methods are more precise but still quite laborious, while the main disadvantage of the ELLE method is that only solvents not miscible with water may be used. Some methods employing pseudoracemic mixtures of guests were also tested. Enantiomers labeled with different chormophores enabled bare eye estimation of enantioselectivity of immobilized guests,[23] while isotope labeling was combined with MS analysis[24−32]

In this work, we present a novel method for the evaluation of chiral recognition–enantioselective liquid–solid extraction (ELSE) − and demonstrate its precision, resistance to experimental errors and high throughput of analysis for a series of chiral carboxylate anion receptors.

Our novel technique relies on the immobilization of a racemic ionic guest ($G_{R,S}$) on an appropriate ion-exchange resin (ℝ). After immersion of the resin in a solute of ions (A), which possess high affinity toward the resin, a replacement reaction takes place eq 5 with its equilibrium shifted to the right.

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$$\mathbb{R}\text{—}G_{R,S} + A_{(sol)} \rightleftharpoons \mathbb{R}\text{—}A + G^{(sol)}_{R,S} \quad (5)$$

Thus, an amount of racemic G is released into the solution. In the presence of a chiral receptor (H*) the guest is bound according to eq 1,2, which affects the equilibrium 5. If the receptor binds G in an enantioselective fashion ($K_S \neq K_R$) the total concentrations[35] of enantiomers of G are not equal ($[G_S]_0 \neq [G_R]_0$), as described in eq 6 (see Supporting Information for details).

$$\frac{[G_S]_0}{[G_R]_0} = \frac{[G_S]_{\mathbb{R}} \cdot (1 + K_S[H^*])}{[G_R]_{\mathbb{R}} \cdot (1 + K_R[H^*])} \quad (6)$$

This equation is simplified if a large excess of resin ($\mathbb{R}\text{—}G_{R,S}$) is used ($[G_S]_{\mathbb{R}} \approx [G_R]_{\mathbb{R}}$). Moreover, if the experiment is carried out with a high concentration of free host (H*) and the association constants (K_S, K_R) are sufficiently high, another approximation is justified ($K[H^*] \gg 1 \Rightarrow 1 + K[H^*] \approx K[H^*]$), resulting in eq 7.

$$\frac{[G_S]_0}{[G_R]_0} \approx \frac{K_S}{K_R} = \alpha \quad (7)$$

This indicates that the enantioselectivity of receptor H* can be determined by a simple analysis of the enantiomeric composition of G extracted into the organic phase. The main advantages of the presented approach are the wide range of applicable solvents and the precise control of the amount of extracted G. This method is still restricted to receptors that form only 1:1 complexes, as the occurrence of species of higher stoichiometry will lead to false results.

Some comments are required on the approximations used to simplify the final equation. The first approximation ($[G_S]_{\mathbb{R}} \approx [G_R]_{\mathbb{R}}$) is always fulfilled when the amount of racemic guest on the resin is about 20 times the amount of A used in the experiment. The second approximation is equivalent to an assumption that nearly all extracted guest is bound by the chiral host. The fraction of extracted guest that is not bound by receptor is a racemate, therefore if a significant amount of guest is unbound the determined enantioselectivity is underestimated. Note that the concentration of H* in the expression (1 + K[H*]) refers to the free host. The concentration of guest in the organic phase is determined by amount of A eq 5; therefore, the chiral host should be present in excess compared to A in order to provide some free H* in the solution. In order to estimate the necessary amount of chiral receptor eq 8 may be used

$$K \cdot [H^*] > z \Leftrightarrow \mathrm{eq}(H^*) > 1 + \frac{z^2}{K[A]_0(1+z)} \quad (8)$$

where eq(H*) designate equivalents of H* calculated against A; $z = 50$ is satisfactory for the approximations. Let us consider the following initial conditions: $\alpha = 1.5$, $[A]_0 = 0.01$ M, $[H\cdot]_0 = 0.02$ M, therefore the concentration of free [H*] is no less than 0.01 M. If the association constants are high ($K > 5000$) the product $K[H^*] > 50$ and the enantioselectivity value can be determined with an error lower than 1%. For K around 1000 ($K[H^*] \approx 10$) the determined α is 1.45 (3% error). For affinities as low as $K \approx 100$ a incorrect value of 1.25 would be obtained. Application of eq 8 indicates that 50 equiv of H* ($[H^*]_0 = 0.5$ M) are necessary in the latter case to obtain reliable results.

We experimentally developed the ELSE method for chiral recognition of carboxylates. We employed mandelate as the model anion, which was bound to the resin in accordance with Scheme 1.

Scheme 1. Binding of the Racemic Guest (Mandelate) to the Ion Exchange Resin

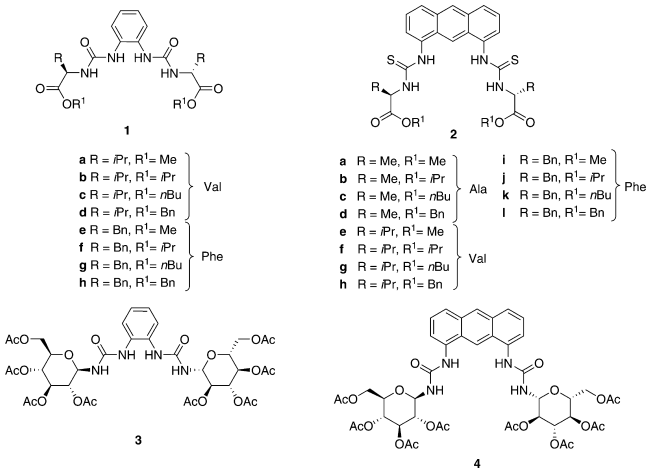
$$\mathbb{R}\text{—Cl} \xrightarrow{\mathrm{NaOH}} \mathbb{R}\text{—OH} \xrightarrow{\mathrm{ManOH_{R,S}}} \mathbb{R}\text{—OMan}_{R,S}$$

$\mathbb{R}$ = Amberlite IRA-400

Man = $C_6H_5CH(OH)CO$–

To validate the methodology, we used a series of neutral anion receptors **1**–**4** recently developed in our group.[16,17,36] These neutral receptors bind anions via multiple hydrogen bonds provided by urea or thiourea groups. All of them were shown to form complexes with carboxylates solely of 1:1 stoichiometry.

Chart 1. Chiral Receptors Used in Validation of the ELSE Methodology



Liberation of mandelate anions into the liquid phase was easily achieved by metathesis reaction with tetrabutylammonium chloride (TBACl), the equilibrium being strongly shifted right because of the high affinity of the resin toward chlorides and the high lipophilicity of mandelate eq 9. Chloride is very useful in such experiments, since our receptors bind chloride much more weakly than carboxylates, thus the presence of chlorides in the liquid phase does not interfere with carboxylate binding.

$$\mathbb{R}\text{—racManO} + \mathrm{TBA} + \mathrm{Cl}^-_{(sol)} \rightleftharpoons \mathbb{R}\text{—Cl} + \mathrm{TBA} + \mathrm{ManO}^-_{(sol)} \quad (9)$$

The enantiomeric composition of $ManO^-$ was determined by GC after the solid phase was filtered off and the anion was transformed into *iso*-propyl ester. GC is very useful in such analysis since it does not require a preceding purification step and enables work to be done on a very small scale. In the initial experiments, we showed that reaction 9 takes place in typical solvents commonly employed in the supramolecular chemistry of anions: chloroform, acetone, acetonitrile, DMF, and DMSO. When a solution of homochiral (*S*)-ManOTBA was treated with a large excess of $\mathbb{R}\text{—OMan}_{R,S}$, after 2 h, a racemate was found in organic phase, indicating that the system indeed

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equilibrates within a short period of time. All further measurements with receptors **1**−**4** were conducted in acetonitrile. In the next series of experiments, the amount of H* (**5e**) was gradually increased from 0 to 4 equiv. (calculated against TBACl) and the enantiomeric composition of extracted mandelate was measured (Figure 1). The collected data were in

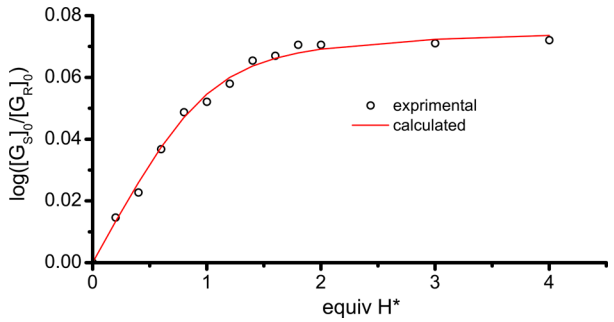

Figure 1. Plot of the ratio of enantiomers vs equivalents of chiral receptor in the system. Data were fitted with a theoretical model, the red line.

excellent conformity with the values calculated according to eqs 3 and 6 (see Supporting Information), demonstrating that the analysis of the system as presented in the aforementioned equilibria and equations is correct. A brief analysis of the plot in Figure 1 indicates that the curve reaches its plateau around 1.5 equiv of H*, from this point the approximation $K[H^*]+1 \approx K[H^*]$ is already met. If the experiment is set up with appropriate excesses of both $\mathbb{R}$—OMan$_{R,S}$ and H*, the result is independent of the exact amount of any of the components used. The direct result of the measurement $\left({}^{[G_S]_0}/_{[G_R]_0} = \alpha\right)$ is, therefore, insensitive to common errors associated with weighting, dissolving, and transferring aliquots of solutions. This also implies that receptors of lower purity may be successfully employed without altering the results.

We performed an experimental validation of the ELSE methodology on a test group of 22 chiral receptors. Five independent measurements, each starting from scratch, performed for receptor **6e** demonstrated the great repeatability of the experiment; the uncertainty of the analysis is estimated to be ±3%. Enantioselectivities obtained in ELSE experiments were compared against the results obtained by competitive titrations (Figure 2).[37] As seen in the graph, taking into account the uncertainties of measurements depicted by error bars, there is a good correlation between the enantioselectivites determined by the two methods. The results are consistent within the experimental errors of the two methods. This serves as additional proof of our concept and indicates that approximations were applied properly. The enantioselectivity values are within the region that is crucial for a perspective CSP (α = 1−1.15) and were properly reproduced in our ELSE method.

A set of 22 receptors can be easily analyzed within 24 h, which includes 8 h of work by a single chemist and overnight autosampled GC analysis. The only steps to be done manually are preparation of solutions of chiral host and TBACl (approximate concentrations), adding resin (approximate amount), and derivatization of extracted anions in the filtrate. In contrast, competitive or direct NMR titrations of such a group of receptors require approximately 10 h of sample

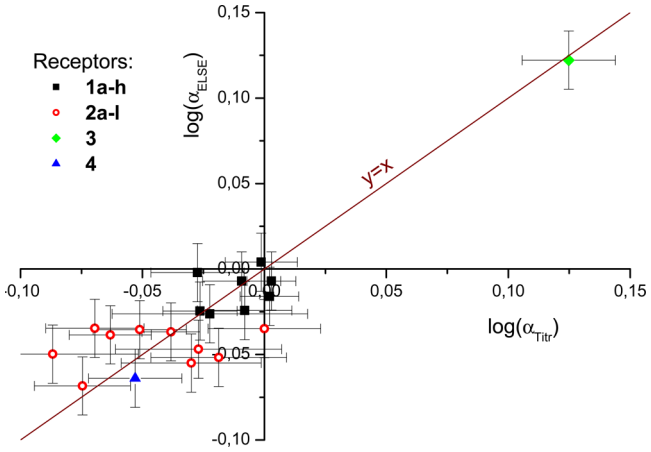

Figure 2. Correlation between enantioselectivities obtained by titrations and ELSE methodology for all tested receptors.

preparation time (weighting, dissolving, transferring) plus at least 40 h of hand operated NMR time.

In the next step, we performed a simultaneous analysis of five enantiomeric pairs of N-acetyl amino acids. In a single experiment the solution of H* and TBACl was stirred with mix of four resins, each modified with one the following amino acids in N-acetyl form: AcVal, AcLeu, AcMet, and AcPhe. Each amino acid was used in an excess as demanded by the aforementioned approximations. In the GC analysis step, we applied a column capable of resolving enantiomers of all natural α-amino acids (Chirasil-Val, Agilent). Results obtained for the mixture were the same as in four separate experiments employing a single amino acid on a resin, while at the same time a 4-fold increase in analysis throughput was attained. The scope of the ELSE method is essentially limited only by the analytical method used for determining the enantiomeric excess of extracted guests.

In this Letter, we have elucidated the principles of the enantioselective liquid−solid extraction (ELSE) method and demonstrated its usefulness. The ELSE technique was tested on a large set of 22 chiral anion receptors and shown to be both accurate and precise, especially in the low selectivities region where the classical methods fail. This methodology makes analyzing the enantioselectivity of binding as simple as analyzing the enantioselectivity of catalytic reactions. It features high throughput and enables fast and accurate screening of libraries of receptors, thus allowing a combinatorial approach to be implemented in future work. We have demonstrated herein that with the aid of the ELSE, the bottleneck in the process of developing enantiodiscriminating receptors is shifted from analysis step to the synthesis step. This method, due to some approximations applied, is yet limited to receptors with high affinities toward guest or not very competitive solvents.

EXPERIMENTAL PROCEDURES

Ion exchange resin (Amberlite 400-IRA) in OH form was prepared from respective Cl form by washing the resin with 5% aqueous NaOH until an aliquot of the eluate added to $AgNO_3$ in diluted HNO_3 resulted in nearly no cloudiness. Next, the resin was washed with distilled water until pH < 9. After it was subsequently washed with methanol, the resin was dried at rt under high vacuum. The loading of functional groups was 1.7 mmol/g.

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Binding of chiral carboxylate (guest) to the resin was achieved by mechanical stirring of a slurry of the resin (5 g, 8.5 mmol) in a solution of an appropriate racemic acid (10 mmol) in methanol (20 mL) for 15 min. The resin was filtered off, washed with methanol (3 × 10 mL) and dried at rt under high vacuum.

Typical extraction experiment. In a 2 mL screw cap vial were placed the following: 10 μmol of chiral host, 5 μmol of tetrabutylammonium chloride (TBACl), 60 mg of resin, and 200 μL of acetonitrile (HPLC grade). The amounts of all components were approximate. The mixture was intensively stirred with a magnetic stirrer for 3 h. The slurry was then filtered through a short pad of Celite (3 mm in a Pasteur pipet stoped with a scrap of cotton wool). The Celite was washed with isopropanol (500 μL) or acetonitrile (500 μL) in case of mandelate or amino acid derivatives as anions, respectively.

Derivatization and GC Analysis. *Experiments with Mandelate.* Mandelate anions were transformed into isopropyl ester by an adaptation of literature method.[33] A solution of 6 M HCl in isopropanol (100 μL) was added to the filtrate, the mixture was heated in a sealed vial at 90 °C for 30 min. It was then cooled to rt, water (500 μL) and *n*-hexane (200 μL) were added, and the mixture was stirred well to extract the ester into upper, hexane phase. An aliquot of the hexane phase was analyzed by GC: Chirasil-Dex-CB (25 m, Agilent); injection volume = 3 μL; split = 8:1; column temperature = 130 °C, isothermal; t_{inj} = 230 °C; FID detector; t_{detec} = 250 °C; t_R = 8.1 min ((*S*)-Man), 8.9 min ((*R*)-Man).

Experiments with N-Acetyl Amino Acids. Amino acid anions were transformed into isopropyl ester by an adaptation of literature method.[34] To the filtrate isopropyl iodide (2 μL, 20 μmol) was added, and the solution was stirred in a sealed vial at 80 °C for 1 h. An aliquot of the solution cooled to rt was analyzed by GC: Chirasil-Val (25 m, Agilent); injection volume = 3 μL; split = 8:1; column temperature = 60 °C for 3 min, ramp 5 °C/min, 120 °C for 5 min; t_{inj} = 230 °C; FID detector; t_{detec} = 250 °C; t_R = 12.2 min (D-Val), 13.6 min (L-Val), 15.4 min (D-Leu), 17.6 min (L-Leu), 24.7 min (D-Met), 25.9 min (L-Met), 27.4 min (D-Phe), 28.4 min (L-Phe).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscombsci.5b00075.

Derivation of equations and results of extraction experiments (PDF)

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Notes

The authors declare no competing financial interest.

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(35) $[G]_0 = [G] + [HG]$, refers to the solution phase
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(37) Logarithm values of enantioselectivity were used because their range is symmetrical with respect of $\alpha = 1$ ($\log(\alpha) = 0$). The range of α is $(0,\infty)$, while the range of $\log(\alpha)$ is $(-\infty, +\infty)$. Opposite selectivities ($\alpha_1 = 1/\alpha_2$) have simply opposite sings of log values ($\log(\alpha_1) = -\log(\alpha_2)$).

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