Chromatographic Properties for Enantiomeric Separation of Amino Acid Derivatives on the Molecularly Imprinted Monoliths

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Since their introduction in 1992 by Fréchet and Svec monolithic supports as stationary phases in high performance liquid chromatography HPLC and capillary electrochromatography CEC have gained significant interest due to a number of unique properties. Their ease of preparation high reproducibility versatile surface chemistry and fast mass transport are advantageous in a variety of applications. Separations in diverse chromatographic modes have been performed in either HPLC or CEC showing their strong point for high-speed separations of biological and synthetic molecules. Although a number of papers have been reported on the application of monolithic supports as chiral stationary phases in CEC and pressure-assisted capillary electrochromatography p-CEC few reports have so far been published on chiral monolithic stationary phases for liquid chromatography.

Molecularly imprinted polymers MIPs as HPLC and CEC stationary phases have been applied to the enantiomeric separation of racemic mixtures due to their unique predetermined selectivity. Traditionally the molecularly imprinted stationary phases in HPLC have been prepared by bulk polymerization. Although the process of bulk polymerization is simple the following steps such as grinding sieving and column packing are tedious time-consuming and not cost-efficient. In alternate approaches molecularly imprinted microspheres have been prepared by suspension polymerization and multi-step swelling polymerization methods. However fairly complicated procedures and reaction conditions are required and the aqueous suspensions used in this technique could interfere with the imprinting and thus lead to a decrease in selectivity. In order to simplify the preparation procedure Matsui et al employed the in-situ polymerization technique to prepare molecularly imprinted monolithic polymer rods in 1993. This type of MIPs exhibited recognition ability for some imprinting molecules such as theophylline nicotine dianisidine cinchona alkaloid and enantiomers of phenylalanine anilide. Subsequently Schweitz and Lin et al used the same approach for the preparation of molecularly imprinted stationary phases to separate racemic mixtures in CEC. Using in-situ polymerization technique MIPs can be synthesized directly inside stainless steel columns or capillary columns without the tedious procedures of grinding sieving and column packing. Furthermore the preparation of this type of MIPs is more cost-efficient as the required amount of template molecules is much lower. However unfortunately the prepared MIPs often suffer from high backpressures and low efficiencies which result in their poor application in practical separation. Especially for the MIPs used in high performance liquid chromatography those problems are more evident. Even though several attempts had been made such as increasing the amount of cyclohexanol in porogenic solvent and adding the latex beads in polymerization mixture to increase the permeability of rod the results

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were still unsatisfactory. We dedicated the optimization of polymerization mixtures and reaction conditions for preparing the molecularly imprinted monolithic stationary phases with both good mass transfer properties and high stereoselectivity for liquid chromatographic separations of chiral compounds. Two enantiomers of amino acid derivatives and diastereomers of cinchona alkaloids had been completely resolved within 6 min on the monolithic stationary phases at elevated flow rates. In addition, the disk columns with monolithic MIP stationary phases were developed for chiral separation under the gradient elution with high flow rate and several enantiomers of amino acid derivatives and diastereomers of cinchona alkaloids had been completely resolved within 3 min with relatively high efficiency.

In this study, in order to extend the application of the in-situ imprinting technique in high-throughput analysis of enantiomer analytes, we further prepared ten molecularly imprinted chiral monolithic stationary phases for studying their chromatographic properties including their separation performance and thermodynamics in chiral separation process.

1 Experimental

1.1 Materials

- N-carbobenzyloxy-L-tryptophan (Cbz-L-Trp)
- N-carbobenzyloxy-DL-tryptophan (Cbz-DL-Trp)
- N-carbobenzyloxy-L-phenylalanine (Cbz-L-Phe)
- N-carbobenzyloxy-D-phenylalanine (Cbz-D-Phe)
- N-carbobenzyloxy-L-tyrosine (Cbz-L-Tyr)
- N-carbobenzyloxy-D-tyrosine (Cbz-D-Tyr)
- N-carbobenzyloxy-L-proline (Cbz-L-Pro)
- N-carbobenzyloxy-D-proline (Cbz-D-Pro)

These materials were obtained from Sigma St. Louis, MO, USA. Fmoc-L-tryptophan, Fmoc-L-Trp, Fmoc-D-Trp, Fmoc-L-phenylalanine, Fmoc-L-Phe, and Fmoc-D-phenylalanine were purchased from Fluka, Buchs, Switzerland. Their structures are shown in Fig. 1. 4-Vinylpyridine and 4-VP from Acros were distilled under vacuum. Ethylene dimethacrylate EDMA from Sigma was extracted with 100 g/L aqueous sodium hydroxide solution and water and dried over anhydrous magnesium sulfate. 2'-Azo-bis isobutyonitrile AIBN and toluene were dried prior to use. All other chemicals and solvents were of analytical or HPLC grade.

![Fig. 1 Structures of template molecules](image-url)

1.2 Preparation of molecularly imprinted monolithic stationary phases

The stationary phase was directly prepared by in-situ polymerization within the confines of a stainless steel chromatographic column tube of 150 × 4 mm i.d. The template molecule free-radical initiator AIBN monomer and cross-linker EDMA were dissolved in porogenic solvents toluene and dodecanol. The compositions are indicated in Table 1. The solution was sonicated for 5 min and deoxygenated with a stream of nitrogen gas for 5 min. The stainless steel tube sealed at the bottom was filled with the above polymerization mixture and then sealed at the top. The polymerization was allowed to proceed at 45 °C for 12 h. After that, the seals were removed, the column was provided with fittings and connected to an HPLC pump and washed exhaustively with methanol/acetic acid 4:1 v/v to remove the porogenic solvents and the template molecules.
1.3 High performance liquid chromatography

A Shimadzu LC-10A HPLC system[a] Shimadzu Kyoto Japan consisting of two LC-10ATvp HPLC pumps and an SPD-10Avp UV-Vis detector was used for all the chromatographic experiments. The data was acquired and processed with WDL-95 chromatographic workstation[c] National Chromatographic R. & A. Center Dalian China[d]. An AT-130 temperature controller[e] Auto-science Tianjin China was used to control the column temperature. The column was washed with mobile phase until a stable baseline was obtained before injection. Acetone was injected as a void marker under corresponding mobile phase. All separations were carried out at ambient temperature[9] except for the studies of the temperature effect on the separation.

The retention times were determined by injection of 10 μg of racemates[e] dissolved in 4 μL of the eluent[f]. Retention factor[7] k was calculated by using the equation $k = \frac{t_R - t_0}{t_0}$ where $t_R$ is the retention time of an analyte and $t_0$ is the elution time of the void marker. Separation factor[6] $\alpha$ was defined as the ratio of the retention factors of enantiomers and used for the evaluation of the selectivity. The resolution factor[9] $R_s$ was calculated according to the method proposed by Wulf et al[10].

2 Results and discussion

2.1 Resolution of enantiomers of amino acid derivatives

As described above[11] the preparation of molecularly imprinted monolithic stationary phases is very simple and the tedious steps in bulk polymerization and suspension polymerization are not required. The procedure of preparation is thereby simplified by the technique compared with the conventional method. Actually[12] some factors must be well considered in the fabrication in order to obtain the optimal products. Fortunately[13] these factors have been investigated in detail in our previous work[14]. By adjusting these factors[15] the optimized preparation conditions of ten molecularly imprinted monolithic stationary phases with amino acid derivatives as template molecules are listed in Table 1. These resulting chiral stationary phases show the resolution ability for the enantiomers of amino acid derivatives[e] and the obtained chromatographic data are listed in Table 2 and the typical chromatograms are shown in Fig. 2. From these results shown in Table 2 and in Fig. 2 it can be clearly seen that most of enantiomers of amino acid derivatives can be well resolved on the corresponding molecularly imprinted monolithic stationary phases. The elution of the imprint molecule[e] as expected[e] is more retarded than that

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>Template molecule</th>
<th>Amount of template molecule/mmol</th>
<th>Porogen mixture</th>
<th>Amount of porogen mixture/mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Cbz-L-Trp</td>
<td>0.278</td>
<td>toluene</td>
<td>1.49/6.51</td>
</tr>
<tr>
<td>M2</td>
<td>Fmoc-D-Trp</td>
<td>0.417</td>
<td>toluene</td>
<td>1.87/6.33</td>
</tr>
<tr>
<td>M3</td>
<td>Fmoc-D-Trp</td>
<td>0.417</td>
<td>toluene</td>
<td>1.87/6.33</td>
</tr>
<tr>
<td>M4</td>
<td>Fmoc-D-Trp</td>
<td>0.557</td>
<td>toluene</td>
<td>0.93/6.78</td>
</tr>
<tr>
<td>M5</td>
<td>Fmoc-D-Trp</td>
<td>0.557</td>
<td>toluene</td>
<td>0.93/6.78</td>
</tr>
<tr>
<td>M6</td>
<td>Cbz-D-Trp</td>
<td>0.417</td>
<td>toluene</td>
<td>1.49/6.51</td>
</tr>
<tr>
<td>M7</td>
<td>Cbz-D-Trp</td>
<td>0.417</td>
<td>toluene</td>
<td>1.49/6.51</td>
</tr>
<tr>
<td>M8</td>
<td>Cbz-L-Pro</td>
<td>0.417</td>
<td>toluene</td>
<td>1.49/6.51</td>
</tr>
<tr>
<td>M9</td>
<td>Boc-L-Trp</td>
<td>0.835</td>
<td>toluene</td>
<td>0.75/6.87</td>
</tr>
</tbody>
</table>

The monomer used in the preparation was 4-VP[h] the amounts of monomer[6] EDMA and AIBN used were 0.835 mmol. 4.19 mmol and 8.75 mg respectively.

The optimized mixture compositions for preparation of molecularly imprinted monolithic stationary phases

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>Analyte</th>
<th>Mobile phase</th>
<th>$k_1$</th>
<th>$\alpha$</th>
<th>$R_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Cbz-DL-Trp</td>
<td>acetonitrile</td>
<td>1.09</td>
<td>2.36</td>
<td>1.67</td>
</tr>
<tr>
<td>M2</td>
<td>Fmoc-DL-Trp</td>
<td>acetonitrile</td>
<td>3.99</td>
<td>2.22</td>
<td>1.76</td>
</tr>
<tr>
<td>M3</td>
<td>Fmoc-DL-Trp</td>
<td>acetonitrile</td>
<td>5.94</td>
<td>1.80</td>
<td>1.40</td>
</tr>
<tr>
<td>M4</td>
<td>Fmoc-DL-Phe</td>
<td>acetonitrile</td>
<td>0.79</td>
<td>2.19</td>
<td>1.32</td>
</tr>
<tr>
<td>M5</td>
<td>Fmoc-DL-Phe</td>
<td>acetonitrile</td>
<td>2.37</td>
<td>1.91</td>
<td>1.46</td>
</tr>
<tr>
<td>M6</td>
<td>Cbz-DL-Tyr</td>
<td>acetonitrile</td>
<td>2.90</td>
<td>1.76</td>
<td>1.51</td>
</tr>
<tr>
<td>M7</td>
<td>Cbz-DL-Tyr</td>
<td>acetonitrile</td>
<td>7.71</td>
<td>1.56</td>
<td>1.42</td>
</tr>
<tr>
<td>M8</td>
<td>Cbz-DL-Phe</td>
<td>acetonitrile</td>
<td>1.81</td>
<td>1.44</td>
<td>0.56</td>
</tr>
<tr>
<td>M9</td>
<td>Cbz-DL-Proc</td>
<td>acetonitrile</td>
<td>0.68</td>
<td>1.23</td>
<td>–</td>
</tr>
<tr>
<td>M10</td>
<td>Boc-DL-Trp</td>
<td>acetonitrile</td>
<td>6.13</td>
<td>1.52</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Conditions[e] the other component in mobile phase is acetic acid[f] flow rate[g] 0.5 mL/min[h] detection wavelengths were 280 nm for Cbz-DL-Trp[h] Fmoc-DL-Trp and Boc-DL-Trp and 258 nm for Fmoc-DL-Phe[i] Cbz-DL-Phe and Cbz-DL-Pro and 276 nm for Cbz-DL-Tyr.
of its optical antipode. Furthermore, no discrimination between enantiomers is observed on the non-imprinted blank column which proves that the chiral recognition sites in the molecularly imprinted monolithic stationary phases are originated from the molecular imprinting process.

In addition, it is found that the selectivity of the molecularly imprinted monoliths prepared is mostly dependent on the structures of the template molecules. The more“ rigid” the structures of template molecules the higher the selectivity of the corresponding molecularly imprinted monoliths obtained. For example, the molecularly imprinted monoliths with Fmoc- andCbz-derived amino acids as template molecules show higher selectivity than those with Boc-derived amino acids which result from the“ rigid” phenyl group in their structures as can be seen in Fig. 1. With respect to Boc-derivative group its structure appears relatively flexible so that its molecular configuration may be difficult to control in the imprinting process which leads to an unstable complex formed in imprinting thereby its recognition capacity decreased. Likewise, when the amino acids with rigid phenyl or indolyl group as template molecules such as Trp Tyr and Phe the obtained molecularly imprinted monoliths showed high selectivity. Among them, the monoliths with the amino acid of Trp as template molecules showed the highest selectivity as the indolyl group in its structure provides more interactive sites than the other amino acids M1 M6 and M8. Similarly, compared with Phe there is an additional hydroxy group in the structure of Tyr which can provide more interactive sites than Phe therefore the selectivity of the monoliths prepared with amino acids of Tyr as template molecule is higher than those with Phe as template molecule. It is consistent with the previous report. Besides the factors mentioned above since the aromatic side chains of amino acid derivatives can also engage in interactions with the system of the functional monomer their rigidity should be also expected to contribute into the selectivity of resulting molecularly imprinted monoliths.

2.2 Study of cross-reactivity

According to the principle of molecular imprinting the selective recognition of the MIPs is mainly originated from the complementarity in shape and the interaction between imprinting molecule and oriented functional groups in the imprinted cavity. This implies that the compounds whose shape and functional groups are similar to those of the template molecule could also interact with the functional groups in cavity and show specific selectivity in the polymer. The phenomenon so-called cross-reactivity is widely observed in molecular imprinting systems which is similar with the cross-reactivity of monoclonal antibody. In this paper the cross-reactivity of six enantiomers of amino acid derivatives on the Cbz-L-Trp imprinted monolith was studied and their separation factors are listed in Table 3. It can be seen that the Cbz-L-Trp imprinted monolith also exhibits the specific selectivity for the tested enantiomers of amino acid derivatives. Among them the separation factors of Cbz-DL-Phe Boc-DL-Trp and Fmoc-DL-Trp are relatively high. The fact is indicative that both the derivative groups of
Cbz and Trp moieties in the molecule of Cbz-DL-Trp have contribution to the recognition process.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Separation factor α/1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cbz-DL-Trp</td>
<td>1.67</td>
</tr>
<tr>
<td>Cbz-DL-Phe</td>
<td>1.29</td>
</tr>
<tr>
<td>Cbz-DL-Tyr</td>
<td>1.09</td>
</tr>
<tr>
<td>Cbz-DL-Pro</td>
<td>1.01</td>
</tr>
<tr>
<td>Boc-DL-Phe</td>
<td>1.13</td>
</tr>
<tr>
<td>Boc-DL-Trp</td>
<td>1.21</td>
</tr>
<tr>
<td>Fmoc-DL-Trp</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Isocratic elution was performed on the M1 stationary phase at 0.5 mL/min with acetonitrile-acetic acid 99.9:0.1 v/v as the mobile phase. Detection wavelengths were the same as in Table 2.

Further analyzing the molecular structures of three amino acid derivatives mentioned above it can be found that Cbz-L-Phe has the most similar structure with Cbz-L-Trp and then Fmoc-L-Trp and Boc-L-Trp in order. So the separation selectivity of Cbz-DL-Phe should be the highest on the Cbz-L-Trp imprinted monolith. Compared with Boc-L-Trp although the Fmoc-L-Trp is more similar with Cbz-L-Trp in structure the separation selectivity of Fmoc-DL-Trp was slightly lower than Boc-DL-Trp as its derivative group of Fmoc might be larger than that of Cbz which led to that Fmoc-L-Trp could not completely match into the recognition cavity in the Cbz-L-Trp imprinted monolith. Therefore its selectivity was reduced. On the contrary the relatively small derivative group of Boc might more easily enter the cavity.

2.3 Thermodynamics of recognition process

In essence the recognition of template molecule by molecular imprinted polymer is originated from their matching in steric structure and functionalities. As far as enantiomers are concerned since their chemical groups are the same their difference is just from their steric structures. The difference leads the two complexes formed between enantiomers and recognition cavity in polymer to show different stabilities in the process of separation. As a result of perfect matching of the template molecule with the recognition cavity the stability of the complex formed between the template molecule and the recognition cavity should be stronger than its counterpart because this is preferential in energy and configuration. Thermodynamic parameters are in charge of the process.

The relationship of complex formation and temperature in enantiomeric recognition process can be given by the Gibbs-Helmholtz equation and Van’t Hoff relation

\[ \Delta G^0 = \Delta H^0 - T \Delta S^0 \]

\[ \ln k = -\Delta H^0 / RT - \Delta S^0 / R + \ln \varphi \]

\[ -\Delta \Delta G^0 = RT \ln \alpha \]

\[ \ln \alpha = -\Delta \Delta H^0 / RT + \Delta \Delta S^0 / R \]

where \( \Delta G^0 \), \( \Delta H^0 \) and \( \Delta S^0 \) are the Gibbs free energy, enthalpy and entropy of two diastereomeric complexes in the process of separation respectively. \( \Delta \Delta G^0 \) \( \Delta \Delta H^0 \) and \( \Delta \Delta S^0 \) are the differences of the Gibbs free energy, enthalpy and entropy of two diastereomeric complexes in the process of separation respectively. \( \varphi \) is phase volume ratio and \( R \) is gas constant. These thermodynamic parameters can be obtained according to the chromatographic retention data from variable-temperature runs. By plotting \( \ln k \) or \( \ln \alpha \) versus \( 1/T \) the linear portions of these plots give \( \Delta H^0 / R \) and \( \Delta S^0 / R \) from the slope and \( \Delta S^0 / R + \ln \varphi \) and \( \Delta \Delta S^0 / R \) from the intercept according to equations 2 and 4. The value of \( \Delta \Delta G^0 \) can be obtained from equation 3 directly.

We determined the thermodynamic parameters in the recognition process of Cbz-DL-Trp Fmoc-DL-Trp and Boc-DL-Trp on the Cbz-L-Trp imprinted monolith in the temperature range of 30 – 60 °C. As seen in Table 4 the absolute values of \( \Delta H^0 \) of the L-forms of these amino acid derivatives are always more than those of the D-forms. It is indicative that the interaction of Cbz-L-Trp imprinted monolith with the L-forms is stronger than with the D-forms. Theoretically the strength of the non-covalent interactions between the functional groups oriented in recognition cavity of molecularly imprinted monolith and template molecule or its analogues depend on the type and distance of interaction. With respect to the enantiomers since they have the same functional groups in structure their types of non-covalent interaction with the molecularly imprinted monolith are identical. So their difference in the strength of interaction with molecularly imprinted monolith is mainly from the steric hindrance and matching difference between enantiomers and the recognition cavity. On the Cbz-L-Trp imprinted
monolith L-form enantiomers could show better matching with the recognition cavity than the D-forms i.e. the L-forms could interact with the functional groups oriented in recognition cavity with the proper distance so the interaction of the imprinted monolith with the L-forms is stronger than that with the D-forms. Furthermore it can also be found that the absolute value of $\Delta H^\circ$ of Cbz-L-Trp is far more than that of the other two L-form amino acid derivatives because only the template molecule could perfectly match its imprinted cavity the molecularly imprinted monolith showed the strongest recognition specificity for it. It further demonstrates that the matching between analytes and recognition cavity plays a very important role in recognition process of the molecularly imprinted polymer. By the way the $\Delta \Delta G^\circ$ value of Cbz-DL-Trp is the lowest among those of three enantiomers of amino acid derivatives shown in Table 4 which also indicates the separation of enantiomers of Cbz-DL-Trp is preferential.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Thermodynamic parameters for enantiomers of amino acid derivatives on their imprinted monolithic columns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte</td>
<td>$\Delta H^\circ$ [kJ/mol]</td>
</tr>
<tr>
<td>Cbz-DL-Trp</td>
<td>$-28.3$</td>
</tr>
<tr>
<td>FMoc-DL-Trp</td>
<td>$-28.5$</td>
</tr>
<tr>
<td>Boc-DL-Trp</td>
<td>$-12.8$</td>
</tr>
</tbody>
</table>

Isocratic elution was performed in mobile phase of acetonitrile-acetic acid [99.7:0.3] v/v.

By the way we have investigated the binding properties of the molecularly imprinted monoliths by frontal analysis method. Dissociation constants of the diastereomers of cinchonine and cinchonidine on the cinchonine imprinted monolith could be determined according to the following equation

$$
\frac{1}{V - V_o} \frac{A}{A_o} = \frac{K_d}{A_o} \frac{A}{A_o} + \frac{1}{B_i}
$$

where $V$ and $V_o$ are the elution volume of analytes and the void volume of monolithic rod respectively. $A/A_o$ is the concentration of analytes applied for frontal analysis. $K_d$ is the dissociation constant between analyte and monolithic MIP and $B_i$ is the effective number of binding sites for interacting with analytes on the monolithic rod. The experimental data were treated according to equation and the results are shown in Fig. 3. The results in Fig. 3 suggest there exist two types of binding sites on the cinchonine imprinted monolith for the imprinted molecule of cinchonine. Their dissociation constants and effective number of binding sites are $0.332$ mmol$^{-1}$, $9.15$ $\mu$mol/g and $1.142$ mmol$^{-1}$, $47.3$ $\mu$mol/g respectively. On the contrary only one type of binding sites was observed for the non-imprinted molecule of cinchonidine with dissociation constant of $0.448$ mmol and the effective number of binding sites of $97.2$ $\mu$mol/g respectively. The heterogeneous distribution of binding sites should be one of the main reasons to result in the peak tailing of template molecule on the molecularly imprinted monolith. We have developed the disk columns with monolithic MIP stationary phases for high-throughput resolution of enantiomers and chiral separation was achieved within 3 min with relatively high efficiency by adopting gradient elution with high flow rate. But in essence we have to further improve the polymerization system for obtaining MIPs with homogeneous surface chemistry and good pore structure thus we will have high-throughput resolution of enantiomers with high efficiency.

3 Concluding remarks

Molecularly imprinted monoliths for template molecules of ten amino acid derivatives have been
prepared as chiral stationary phases for enantiomeric separation in liquid chromatography. Most of the enantiomers of amino acids derivatives can be successfully resolved on the corresponding molecularly imprinted monolithic stationary phases. Meanwhile it is found that the selectivity on the prepared molecularly imprinted monoliths is mostly dependent on the structures of the template molecules. The more “rigid” the structures of template molecules, the higher the selectivity of the corresponding molecularly imprinted monolith obtained. Furthermore it is indicative that both derivative groups of Cbz and Trp moieties in the Cbz-DL-Trp have contribution to the recognition process by studying the cross-reactivity of six enantiomers of amino acid derivatives on the Cbz-L-Trp imprinted monolith. In addition, the thermodynamic investigations further demonstrate that the matching between analytes and recognition cavity plays a very important role in the recognition process of molecularly imprinted polymer. However only the template molecule can perfectly match its imprinted cavity.

References

[16] Lämmerhofer M, Tobler E, Zarbl E, Lindner W, Svec F.
Fréchet J M J. Electrophoresis 2003; 24: 2986
[37] Sellergren B, J Chromatogr A 1994; 673: 133
[38] Schweitz L, Andersson L I, Nilsson S, J Chromatogr A 1997; 792: 401
[48] Spivak D A, Campbell J, Analyst 2001; 126: 793
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In the past 20 years Prof. ZOU has been working on the research topics including capillary electrophoresis and electrophoresis chromatography chromatographic media for purification and analysis of biopolymers and enantiomers screening and analysis of bioactive compounds in traditional Chinese medicines by biochromatography comprehensive 2-dimensional HPLC and novel matrices for MALDI-TOF MS etc. He has published about 200 scientific papers totally and among them about 120 papers and 6 review papers were published in the international journals. He services as a member of editorial board for the Chin. J. Anal. Chem. Chin. J. Chromatogr. Chin. Sci. Bull. Chin. J. Environ. Chem.

He has received number of awards such as Excellent Young Scientist awarded by the Chinese Association for Science and Technology in 1990 Silver Award for Excellent Young Scientist by Chinese Academy of Science in 1991 Third-Class Award for Natural Sciences by CAS in 1996 Excellent Young Scientist Funding awarded by the National Natural Science Foundation of China in 1997 Excellent Young Scientist Award by the QiuShi Foundation HK in 1998 First-Class Award for Natural Sciences by Liaoning Province in 2002.

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