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Research Article

Recognizing Amino Acid Chirality with Surface-Imprinted Polymers Prepared in W/O Emulsions

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A molecularly imprinted polymer was prepared by a surface molecular imprinting technique in water-in-oil (W/O) emulsion. In this technique, the solid polymer, which is molecularly imprinted at the internal cavity surface, is prepared by polymerizing W/O emulsions consisting of a water-soluble imprinted molecule, a functional host molecule, an emulsion stabilizer, and a crosslinking agent. Dioleoyl phosphate was used as an emulsion stabilizer, and this compound also acted as a monomer and a host functional group in the imprinted cavity. Divinylbenzene was used as a crosslinker. Tryptophan methyl ester and phenylalanine methyl ester were used as the target template materials. These imprinted polymers exhibited enantiomeric selectivity in absorption experiments, and the maximum separation factor was 1.58. The enantiomeric selectivity with tryptophan methyl ester was higher than that with phenylalanine methyl ester.

1. Introduction

Recently, significant attention has been paid to the development of a molecular imprinting technique that enables polymers to mimic biological receptors. This technique is a very useful approach for the fabrication of a matrix with molecular recognition sites, which are formed by the addition of template molecules during the matrix formation process and the removal of the template molecule after the matrix formation [1–5]. Polymers that were prepared by the molecular imprinting technique have attracted much attention as interesting separation tools, especially for high performance liquid chromatography (HPLC). The imprinting technique is conceptually easy to apply to a wide variety of target molecules. Important applications are optical resolutions of amino acids or amino acid derivatives [6–12], direct enantiomeric separation of drugs [13, 14], and separation of sugar or sugar derivatives [4, 15, 16].

The "surface molecular imprinting technique" was proposed to overcome the inapplicability to water-soluble substances, which are important in the biological or biomedical field [17–20]. In this technique, the solid polymer, which is molecularly imprinted at the internal cavity surface, is prepared by polymerizing water-in-oil (W/O) emulsions consisting of a water-soluble imprint molecule, a functional host molecule, an emulsion stabilizer, and a crosslinking agent. The organic-aqueous interface in W/O emulsions is utilized as the recognition field for a target molecule. The target molecule forms a complex with the functional host molecule, while the orientation of the functional host molecule itself is fixed at the oil-water interface. After polymerization, preparation provides the recognition sites at the inner cavity surface of the imprinted bulk polymer. The complex between the functional host molecule and the target material should not be too hydrophobic or hydrophilic, because otherwise the complex would not be located at the oil-water interface. Thus, a functional host molecule should be amphiphilic, just like a surfactant molecule, in order to yield a high template effect for the target molecule. The crosslinking agent crosslinks the organic phase and stabilizes the water pool or the imprinted water cavity after polymerization. The bulk polymer that is obtained is ground to small particles in order to interact with the target molecules in the solution.

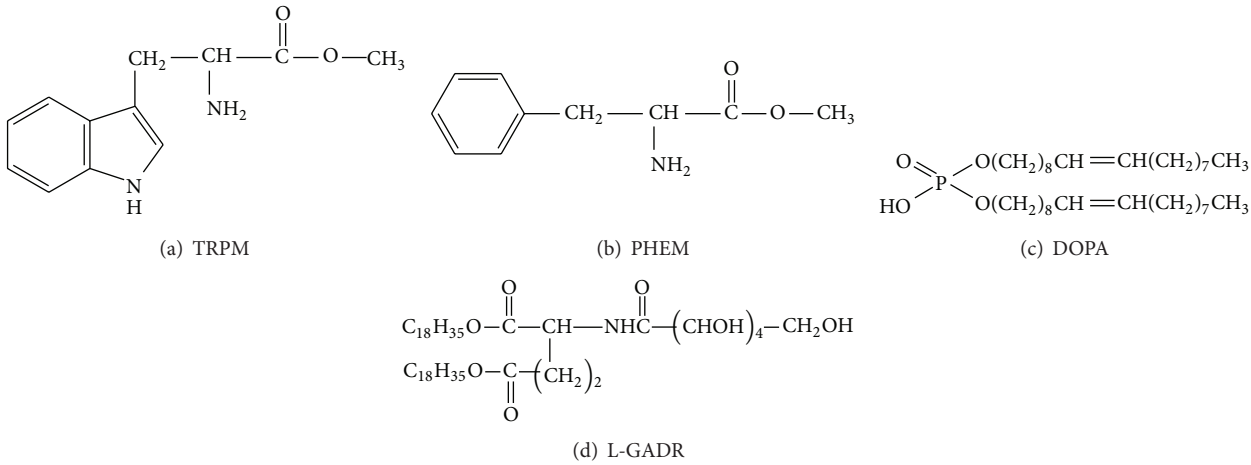

Figure 1: Molecular structures of (a) TRPM, (b) PHEM, (c) DOPA, and (d) L-GADR.

In this study, we tried to have a host molecule with a crosslinker participate in the polymerization. So dioleoyl phosphoric acid was selected as a host molecule, and the polymerization was conducted with a large amount of benzoyl peroxide at a high temperature to polymerize both the host molecule and divinylbenzene.

2. Experimental

2.1. Reagents and Instruments. L-Tryptophan methyl ester (L-TRPM), D-tryptophan methyl ester (D-TRPM), divinylbenzene (DVB), L-phenylalanine methyl ester (L-PHEM), D-phenylalanine methyl ester (D-PHEM), benzoyl peroxide (BPO), L-glutamic acid, oleyl alcohol, D-gluconic acid δ-lactone, phosphorus oxychloride, styrene, and xylenes were obtained from Aldrich. Dioleyl phosphoric acid (DOPA) and L-glutamic acid dioleylester ribitol (L-GADR) were synthesized using already reported methods [17, 21–23]. Divinylbenzene was used after treatment with silica gel to remove an inhibitor. Figure 1 shows the chemical structures of TRPM, PHEM, DOPA, and L-GADR.

Infrared spectroscopy (IR) was performed using an FT-IR 680 (Jasco International). Sonication was performed using a Cole-Parmer 4710250W sonicator. Scanning electron microscopy (SEM) was performed using both a Hitachi S-2500C and a Hitachi S-5200V scanning electron microscope.

2.2. Preparation of L- or D-TRPM-Imprinted Polymers. 40.0 g (0.307 mol) of DVB and 1.50 g (2.50 mmol) of DOPA were dissolved in 20 mL of xylenes. Another solution was prepared by dissolving 0.102 g (0.40 mmol) of L- or D-TRPM·HCl in 20 mL of water which contained 0.10 M phosphate buffer solution adjusted to pH 7.0. This buffer solution was added to the xylenes solution. The mixture was sonicated for 5 min to obtain the stable W/O emulsion. Then, 1.60 g (6.61 mmol) of BPO initiator was added to this solution, and then, this solution was sonicated again for 2 min. This mixture was polymerized at 60°C for 30 min, and then, temperature was gradually raised to 140°C, and the reaction was continued at 140°C for 24 h under a flow of nitrogen. The obtained bulk polymer was dried under a vacuum at 50°C for 48 h and ground into particles. The particles were washed with 0.50 M HCl to remove the imprinted L- or D-TRPM and then filtered off. This procedure was repeated several times until the imprinted molecule in the filtrate could not be detected by a UV spectrometer. Finally, the polymer was dried under a vacuum at 50°C for 48 h.

2.3. Preparation of L- or D-PHEM-Imprinted Polymers. These imprinted polymers were prepared by the same method as the preparation for the L- or D-TRPM-imprinted polymers using L- or D-PHEM instead of L- or D-TRPM.

2.4. Adsorption Experiments Using the Imprinted Polymers. The batchwise adsorption experiments of L,D-TRPM and L,D-PHEM were conducted for the L- or D-TRPM-imprinted polymers and L- or D-TRPM-imprinted polymers, respectively. 0.050 g of the imprinted polymer was added to 5.0 mL of aqueous solution containing 0.50 mM of L- or D-TRPM and placed in a sealed 10 mL tube. The pH was adjusted to a desired value between 3.0 and 8.0 with 0.10 M KH_2PO_4 or K_2HPO_4 and 0.10 M HNO_3 or 0.10 M NaOH. The mixture was shaken in a thermostated water bath at 30°C for 24 h. The polymers were then filtered off through a polyethylene membrane. The amount of each amino acid derivative adsorbed to the polymers was calculated from their residual amount in the filtrate. The concentration of amino acid derivatives was analyzed by an HPLC system. The adsorption tests were conducted at least 3 times, and the data was analyzed and compared with average values. The experimental errors were less than 6%.

2.5. Binding Constant of the Substrate for Imprinted Polymer. Binding constants of the substrate for the imprinted polymer were evaluated with the batchwise method. 0.050 g of the imprinted polymer sample was immersed in a sealed 10 mL tube. Then, a 5 mL of the aqueous solution was added, which was buffered with 0.10 M KH_2PO_4 or K_2HPO_4 and 0.10 M

HNO_3 or 0.10 M NaOH containing a substrate that was adjusted to a desired concentration of between 0.050 M and 1.0 M. The mixture was shaken at 30°C for 24 h. The polymers were then filtered off through the polyethylene membrane. The concentration of the substrate in the filtrate was analyzed by means of the HPLC system. The binding constants were calculated by a modified Scatchard equation [24, 25].

3. Results and Discussion

3.1. The Preparation of the Imprinted Polymer. The imprinted polymers were prepared by the surface molecular imprinting technique with W/O emulsions. L- or D-TRPM and L- or D-PHEM were used as the target imprinted molecules, and DOPA was used as both a functional monomer and an emulsion stabilizer. DVB was used as a crosslinker, xylenes were used as a diluent, and BPO was used as an initiator. In order to determine the polymerization conditions, we referred to the result of the radical polymerization for triglyceride oils. Many researchers attempted the radical polymerization of the triglyceride oils with styrene or DVB [26, 27]. Therefore, we used a larger amount of initiator compared to the general polymerization. BPO was used at 2.2 mol % versus the amount of DVB, and this amount is 2.6 times larger than the amount of DOPA. The polymerization was conducted at first at 60°C for 30 min to prevent bumping, and after that the temperature was raised to 140°C gradually, and the polymerization was continued for 24 h under a flow of nitrogen at 140°C. In order to conduct the experiment at 140°C, the xylenes (bp 137–140°C) were used as a diluent.

In order to estimate the exact amount of DOPA which participated in the polymerization, we conducted the preliminary experiment for the polymerization of DOPA with styrene or DVB. 31.9 g (0.307 mol) of styrene and 1.50 g (2.50 mmol) of DOPA were dissolved in 20 mL of xylenes, and the polymerization was conducted with 1.60 g (6.61 mmol) of BPO initiator at 140°C for 24 h under a flow of nitrogen. After finishing the polymerization, the remaining unpolymerized amount of DOPA was estimated using HPLC. The result showed that 56% of DOPA remained in the filtrate. This means that 44% of the DOPA was participated in the polymerization. We also tried another preliminary experiment with DVB. Instead of styrene, 40.0 g (0.307 mol) of DVB was used and the same experiment was conducted, and the remaining amount of DOPA was estimated using HPLC. The results showed that 9% of the DOPA remained in the filtrate. This means that 91% of DOPA stayed in the crosslinked polymer. We did not think that all 91% of the DOPA was polymerized with DVB. The IR spectrum of this polymer showed that some amount of double bonds still remained in the polymer. Because only 44% of the DOPA participated in the polymerization with styrene, we presumed that more than 44% of the DOPA participated in the polymerization with DVB. Therefore, we also presumed that some small part of the DOPAs were possible to anchor onto the highly crosslinked polymer.

After polymerization, the obtained polymer was dried under vacuum, and this polymer was grounded into small particles. After that, the imprinted molecules in the polymer were extracted with 0.50 M HCl solution. The obtained polymer particles were dried under vacuum. The average size of the particles was about 50 um.

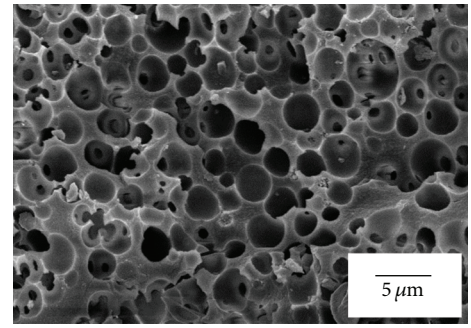

Figure 2: SEM photograph of L-TRPM-imprinted polymer.

Figure 2 shows the SEM of the particle surface. Figure 2 shows that a lot of round shape rooms were formed by the W/O emulsions. At the surface of these round shape rooms, numerous imprinted cavities were positioned.

3.2. Adsorption Behavior of the Imprinted Polymers. The adsorption experiment was conducted using the L-TRPM-imprinted polymer. The enantiomeric selectivity of this imprinted polymer was estimated using L-TRPM and D-TRPM as substrates. We conducted the adsorption experiment at pH 3.0, pH 5.0, pH 7.0, and pH 8.0. We quantitatively characterized the template effect in the L- and D-imprinted polymers by evaluating the binding constants. The binding constant (K) can be evaluated on the basis of the slope and intercepted by the modified Scatchard plot. The binding constant becomes an indicator to express an adsorption affinity of recognition sites for the target amino acid derivative. To discuss the enantioselectivity quantitatively, we defined the separation factor as follows: $\alpha = K_L/K_D$ or $\alpha = K_D/K_L$.

Table 1 shows the results for the adsorption of L-TRPM and D-TRPM on the L-TRPM-imprinted polymer. The results showed that the binding constant increased as the pH increased. The separation factor was 1.02 at pH 3.0. This means that there was not much difference in the binding constant of the L-TRPM with that of the D-TRPM, but the separation factor was 1.58 at pH 7.0. This means that this imprinted polymer had a high enantiomeric selectivity at pH 7.0. At pH 8.0, the separation factor decreased relative to the data at pH 7.0. This result indicates that the interaction of the functional group in the imprinted cavity with the functional group of the substrate became maximized at pH 7.0.

Using the D-TRPM-imprinted polymer, almost similar results were obtained compared to the data from the L-TRPM-imprinted polymer. Table 2 shows the results for the adsorption of L-TRPM and D-TRPM on the D-TRPM-imprinted polymer. The separation factor was a little lower, but the difference value is within the error range.

The adsorption experiment was conducted using the L-PHEM-imprinted polymer. The enantiomeric selectivity of this imprinted polymer was estimated using L-PHEM and D-PHEM as the substrates. Table 3 shows the results for

Table 1: The adsorption of L-TRPM and D-TRPM on the L-TRPM-imprinted polymer.

pH	Substrate	Binding constant, K (M^{-1})	Separation factor, α
3.0	L-TRPM	1.29×10^2	1.02
	D-TRPM	1.27×10^2	
5.0	L-TRPM	1.02×10^3	1.12
	D-TRPM	0.91×10^3	
7.0	L-TRPM	3.05×10^3	1.58
	D-TRPM	1.93×10^3	
8.0	L-TRPM	3.48×10^3	1.39
	D-TRPM	2.50×10^3	

Table 2: The adsorption of L-TRPM and D-TRPM on the D-TRPM-imprinted polymer.

pH	Substrate	Binding constant, K (M^{-1})	Separation factor, α
3.0	L-TRPM	1.32×10^2	1.04
	D-TRPM	1.37×10^2	
5.0	L-TRPM	0.94×10^3	1.11
	D-TRPM	1.04×10^3	
7.0	L-TRPM	2.02×10^3	1.49
	D-TRPM	3.01×10^3	
8.0	L-TRPM	2.55×10^3	1.32
	D-TRPM	3.36×10^3	

Table 3: The adsorption of L-PHEM and D-PHEM on the L-PHEM-imprinted polymer.

pH	Substrate	Binding constant, K (M^{-1})	Separation factor, α
3.0	L-PHEM	1.48×10^2	1.04
	D-PHEM	1.42×10^2	
5.0	L-PHEM	1.72×10^3	1.06
	D-PHEM	1.62×10^3	
7.0	L-PHEM	3.45×10^3	1.35
	D-PHEM	2.56×10^3	
8.0	L-PHEM	4.17×10^3	1.18
	D-PHEM	3.53×10^3	

the adsorption of L-PHEM and D-PHEM on the L-PHEM-imprinted polymer, and Table 4 shows the results for the adsorption of L-PHEM and D-PHEM on the D-PHEM-imprinted polymer. The results showed that the trend of the data was almost similar with that of the TRPM-imprinted polymer, but the separation factor was much lower in value compared with that of the TRPM-imprinted polymer. The separation factor of the L-TRPM-imprinted polymer was 1.58 at pH 7.0 but the separation factor of the L-PHEM-imprinted polymer was 1.35. We think that the nitrogen in the tryptophan ring of TRPM participates in an important interaction between the functional group in the imprinted cavity and the functional group in the substrate that is needed for increasing enantiomeric selectivity.

3.3. Effect of the Chiral Emulsion Stabilizer. The Goto group introduced the surface molecular imprinting technique. They used L-GADR as an emulsion stabilizer [28]. So, we tried to use this chiral compound in our system. 0.207 g (0.251 mmol) of L-GADR was added during the preparation of the imprinted polymer with L-TRPM as a template molecule. The results showed that the separation factor was 1.60 at pH 7.0. We tried the same experiment 3 times. All of the results ((1) 1.63, (2) 1.58, and (3) 1.59) showed that the separation factor was more than 1.58. This means that L-GADR was effective in achieving enantiomeric selectivity, but compared to the result without L-GADR, the separation factor was bigger by a small amount. We also tried to conduct the experiment using 2 times the amount of L-GADR (0.414 g, 0.502 mmol). The results showed that the separation factor was average 1.61 ((1) 1.58, (2) 1.63, and (3) 1.62). This result value shows a little increase, but the value is within the experimental error. So, we concluded that this chiral compound is not very effective for achieving enantiomeric selectivity.

Table 4: The adsorption of L-PHEM and D-PHEM on the D-PHEM-imprinted polymer.

pH	Substrate	Binding constant, K (M^{-1})	Separation factor, α
3.0	L-PHEM	1.39×10^2	1.04
	D-PHEM	1.45×10^2	
5.0	L-PHEM	1.52×10^3	1.08
	D-PHEM	1.64×10^3	
7.0	L-PHEM	2.64×10^3	1.33
	D-PHEM	3.51×10^3	
8.0	L-PHEM	3.50×10^3	1.16
	D-PHEM	4.06×10^3	

4. Conclusion

Amino acid-imprinted polymers were prepared by a surface imprinting technique in a W/O emulsion. DOPA was used as the emulsion stabilizer and the material for the host functional group in the imprinted cavity. DVB was used as a crosslinker. TRPM and PHEM were used as the imprinted materials and the substrate materials. The absorption experiments were conducted using these imprinted polymers with enantiomeric substrate materials. These polymers exhibited high enantiomeric selectivity for TRPM and PHEM. The separation factor of the TRPM-imprinted polymer was much higher than that of the PHEM-imprinted polymer.

Conflict of Interests

All of the authors do not have a direct financial relation with Aldrich, Jasco International, Cole-Parmer, and Hitachi.

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References

[1] R. Gupta and A. Kumar, "Molecular imprinting in sol-gel matrix," *Biotechnology Advances*, vol. 26, no. 6, pp. 533–547, 2008.

[2] A. G. Mayes and K. Mosbach, "Molecularly imprinted polymer beads: suspension polymerization using a liquid perfluorocarbon as the dispersing phase," *Analytical Chemistry*, vol. 68, no. 21, pp. 3769–3774, 1996.

[3] S. W. Lee, D. H. Yang, and T. Kunitake, "Regioselective imprinting of anthracenecarboxylic acids onto TiO_2 gel ultrathin films: an approach to thin film sensor," *Sensors and Actuators B*, vol. 104, no. 1, pp. 35–42, 2005.

[4] D. H. Yang, N. Takahara, S. W. Lee, and T. Kunitake, "Fabrication of glucose-sensitive TiO_2 ultrathin films by molecular imprinting and selective detection of monosaccharides," *Sensors and Actuators B*, vol. 130, no. 1, pp. 379–385, 2008.

[5] D. H. Yang, Y. R. Ham, M. H. Oh et al., "Simple method for the fabrication of 1-hydroxypyrene-imprinted TiO_2 gel nanofilms," *Current Applied Physics*, vol. 9, no. 2, pp. e136–e139, 2009.

[6] B. Sellergren, M. Lepistö, and K. Mosbach, "Highly enantioselective and substrate-selective polymers obtained by molecular imprinting utilizing noncovalent interactions. NMR and chromatographic studies on the nature of recognition," *Journal of the American Chemical Society*, vol. 110, no. 17, pp. 5853–5860, 1988.

[7] L. I. Andersson, D. J. O'Shannessy, and K. Mosbach, "Molecular recognition in synthetic polymers: preparation of chiral stationary phases by molecular imprinting of amino acid amides," *Journal of Chromatography*, vol. 513, pp. 167–179, 1990.

[8] K. J. Shea, D. A. Spivak, and B. Sellergren, "Polymer complements to nucleotide bases. Selective binding of adenine derivatives to imprinted polymers," *Journal of the American Chemical Society*, vol. 115, no. 8, pp. 3368–3369, 1993.

[9] O. Ramström, I. A. Nicholls, and K. Mosbach, "Synthetic peptide receptor mimics: highly stereoselective recognition in non-covalent molecularly imprinted polymers," *Tetrahedron Asymmetry*, vol. 5, no. 4, pp. 649–656, 1994.

[10] M. Kempe, "Antibody-mimicking polymers as chiral stationary phases in HPLC," *Analytical Chemistry*, vol. 68, no. 11, pp. 1948–1953, 1996.

[11] C. Yu and K. Mosbach, "Molecular imprinting utilizing an amide functional group for hydrogen bonding leading to highly efficient polymers," *Journal of Organic Chemistry*, vol. 62, no. 12, pp. 4057–4064, 1997.

[12] S. Vidyasankar, M. Ru, and F. H. Arnold, "Molecularly imprinted ligand-exchange adsorbents for the chiral separation of underivatized amino acids," *Journal of Chromatography A*, vol. 775, no. 1-2, pp. 51–63, 1997.

[13] L. Fischer, R. Müller, B. Ekberg, and K. Mosbach, "Direct enantioseparation of β-adrenergic blockers using a chiral stationary phase prepared by molecular imprinting," *Journal of the American Chemical Society*, vol. 113, no. 24, pp. 9358–9360, 1991.

[14] L. I. Andersson, "Application of molecular imprinting to the development of aqueous buffer and organic solvent based radioligand binding assays for (S)-propranolol," *Analytical Chemistry*, vol. 68, no. 1, pp. 111–117, 1996.

[15] G. Wulff and S. Schauhoff, "Racemic resolution of free sugars with macroporous polymers prepared by molecular imprinting. Selectivity dependence on the arrangement of functional groups versus spatial requirements," *Journal of Organic Chemistry*, vol. 56, no. 1, pp. 395–400, 1991.

[16] G. Wulff and J. Haarer, "Enzyme-analogue built polymers, 29. The preparation of defined chiral cavities for the racemic resolution of free sugars," *Die Makromolekulare Chemie*, vol. 192, no. 6, pp. 1329–1338, 1991.

[17] K. Uezu, H. Nakamura, J. I. Kanno, T. Sugo, M. Goto, and F. Nakashio, "Metal ion-imprinted polymer prepared by the combination of surface template polymerization with postirradiation by γ-rays," *Macromolecules*, vol. 30, no. 13, pp. 3888–3891, 1997.

[18] M. Yoshida, K. Uezu, F. Nakashio, and M. Goto, "Spacer effect of novel bifunctional organophosphorus monomers in metal-imprinted polymers prepared by surface template polymerization," *Journal of Polymer Science A*, vol. 36, no. 15, pp. 2727–2734, 1998.

[19] M. Yoshida, K. Uezu, M. Goto, and S. Furusaki, "Required properties for functional monomers to produce a metal template effect by a surface molecular imprinting technique," *Macromolecules*, vol. 32, no. 4, pp. 1237–1243, 1999.

[20] M. Yoshida, K. Uezu, M. Goto, and S. Furusaki, "Metal ion imprinted microsphere prepared by surface molecular imprinting technique using water-in-oil-in-water emulsions," *Journal of Applied Polymer Science*, vol. 73, no. 7, pp. 1223–1230, 1999.

[21] M. Goto, M. Matsumoto, K. Kondo, and F. Nakashio, "Development of new surfactant for loquid surfactnat membrane process," *Journal of Chemical Engineering of Japan*, vol. 20, no. 2, pp. 157–164, 1987.

[22] K. Uezu, H. Nakamura, M. Goto et al., "Novel metal ion-imprinted resins prepared by surface template polymerization with W/O emulsion," *Journal of Chemical Engineering of Japan*, vol. 27, no. 3, pp. 436–438, 1994.

[23] M. Goto, K. Kondo, and F. Nakashio, "Acceleration effect of anionic surfactants on extraction rate of copper with liquid surfactant membrane containing LIX65N and nonionic surfactant," *Journal of Chemical Engineering of Japan*, vol. 22, no. 1, pp. 79–84, 1989.

[24] B. Sellergren, "Molecular imprinting by noncovalent interactions. Enantioselectivity and binding capacity of polymers prepared under conditions favoring the formation of template complexes," *Die Makromolekulare Chemie*, vol. 190, no. 11, pp. 2703–2711, 1989.

[25] J. Mathew and O. Buchardt, "Molecular imprinting approach for the recognition of adenine in aqueous medium and hydrolysis of adenosine 5′-triphosphate," *Bioconjugate chemistry*, vol. 6, no. 5, pp. 524–528, 1995.

[26] F. S. Güner, Y. Yağci, and A. T. Erciyes, "Polymers from triglyceride oils," *Progress in Polymer Science*, vol. 31, no. 7, pp. 633–670, 2006.

[27] O. S. Kabasakal, "Styrenation of mahaleb and anchovy oils," *Progress in Organic Coatings*, vol. 53, no. 3, pp. 235–238, 2005.

[28] M. Yoshida, K. Uezu, M. Goto, and S. Furusaki, "Surface imprinted polymers recognizing amino acid chirality," *Journal of Applied Polymer Science*, vol. 78, no. 4, pp. 695–703, 2000.