CHAPTER V

CONCLUSION

The main objective of this research is to study the use of MIPs for selective extraction of genistein from soybean. Consequently, the MIPs selective to genistein were investigated. MIPs are commonly generated by using the target analyte as a template molecule. Nevertheless, for the limited amount of the target compounds such as genistein, the alternative imprinting approach is required. In this study, the fragment imprinting approach which is the synthesis of MIPs using the fragment templates of genistein was studied. The best performing MIPs was selected to apply for extraction of soybean isoflavones from natural samples.

MIPs were prepared using chromone (I), phloroglucinol (II) and 4-hydroxyphenylacetic acid (III) as the fragment templates. All of the MIPs were synthesized by bulk polymerization using ACN and EGDMA as a porogen and cross-linker, respectively. The various functional monomers were used which were AA, MAA and 4-VP. The template : functional monomer : cross-linker ratio was 1:4:20.

The binding performance of the synthesized polymers was investigated with both their corresponding templates and genistein. It was found that PIII-VP showed the good binding performance with both its template and genistein among the synthesized polymers. Therefore, this polymer was then evaluated to use as a sorbent in MISPE for isolation of genistein from soybean extracts. An aliquot amount of genistein was loaded into MISPE cartridge and the extraction conditions in MISPE, including loading, washing and elution conditions, were optimized. The optimum MISPE condition which gave the highest genistein recovery was loading of genistein with 40% MeOH in water, washing with 20% ACN in water containing 0.1% FA and eluting with 1% FA in ACN. Nevertheless, there is almost no different in genistein recovery between MIP and NIP. This result suggesting that binding of genistein to the polymer is occurred through non-specific interaction.

After extraction conditions were optimized, the selectivity of MISPE process was investigated. Quercetin was selected as the structurally related flavonoids in the selectivity study. It was found that PIII-VP showed the highest selectivity toward genistein resulting from the template effect.

Furthermore, the combination of the representative MIPs from each fragment templates as mixed-mode MISPE was studied to improve the specificity and selectivity of the MISPE for genistein extraction. Therefore, the selectivity study was investigated in the same way as the single mode MISPE. It was observed that the mixed-mode gave the lower selectivity (ε) in comparison to the single mode while the specificity (α′) was slightly better. The selected protocol was applied for selective extraction of genistein from soybean using both single (PIII-VP) and mixed-mode MISPE (PI-II-III). The obtained results showed that the MISPE procedure can remove some interferences. The soybean extract can be partially cleaned up after both single and mixed-mode MISPE processes. However, the cross-reactivity was observed when using quercetin, the structurally related compound, as test substrate. The purity of genistein after MISPE process was slightly better than before MISPE process. It has been reported that the metal coordination interaction is more like a covalent interaction than hydrogen bonding or electrostatic interactions in terms of strength, specificity and directionality.[71] These features make metal coordination a promising binding mode to prepare highly specific recognition polymers. Therefore, the appropriate imprinting approach which gave the high specificity towards its template as mentioned above and MISPE protocol should be re-investigated to improve the specificity of binding site generation which lead to more efficient MISPE.