CHAPTER IV

CONCLUSIONS

The main purpose of this research was to synthesize MIPs for NVP detection. In this work, to obtain MIPs with high specificity, various parameters that can influence properties of MIPs including type and ratio of functional monomers, cross-linkers and porogens were investigated. Due to limited material of NVP, NAM, the structural related compound was chosen as a template molecule in a primary screening. UV binding studies were used to screen the eleven synthesized polymers (P1-P11). It was found that P11 synthesized by precipitation polymerization method (MAA:TRIM, 1:1) demonstrated to be the best polymer. This result suggested that MAA and TRIM are good candidates in the preparation of MIPs for NVP. The polymers (P12-P16) were therefore synthesized and screened for their binding efficiency. The UV binding showed that polymer P15 has the best binding characterizations in both aqueous buffer and organic solvent.

Selected polymers were characterized for morphology, loading and selectivity. The morphology of the polymer was investigated by SEM technique. SEM image showed that microspheres particle of P15 has average diameter of 100-200 μm in comparison to those ground particles from bulk polymerization (P13) having average diameter of 5-15 μm. The maximum number of binding sites (Q_{max}) and dissociation constant (K_d) were determined by Scatchard analysis. The Q_{max} and K_d values were calculated to be 57.92 μg/mg and 134.27 mg/ml for P11 and 95.62 μg/mg and 166.72 mg/ml for P15. The selectivities of P11 and P15 were later evaluated by UV binding of

a series of structurally related compounds of NVP including NAM, BAM, 2-Apy, 3-Apy, 4-Apy, pyridine, aniline and OPD. Interestingly, polymer P11 exhibited high selectivity for NVP whereas P15 bind to NVP and NAM almost equally. This may be due to structural similarity of NVP and NAM in which NAM structure is actually part of NVP structure.

Potential application of P11 and P15 in the development of MIAs for NVP detection was also investigated. After a series of parameter optimization including varying pH, % of additive surfactant, amount of polymer and dilution of enzyme labeled analyte, two conditions were performed. The high dilution of probe used in the first condition was unable to obtain a calibration curve for the competitive experiment. In the other system, the suitable condition was performed using high concentration of NVP-HRP conjugate. This method obtained the calibration curves for detection of NVP at concentrations ranging from 10-300 and 100-400 μg/ml using P11 and P15, respectively.

In summary, MIPs that exhibit high binding selectivity to NVP were synthesized and were shown to be potentially useful in the development of a cheap, reliable and rapid assay for NVP detection. Especially, the binding of NVP can be performed with high efficiency in aqueous medium. With these advantages, the obtained MIPs can be developed in the other applications of imprinting technique such as solid phase extraction.

In continuation of the work described in this thesis, the following suggestions for further work are made:

1). In this work, enzyme activity was measured in the supernatant which was different from most immunoassays where the bound analyte-enzyme conjugate is

quantified. Thus, the MIPs may be prepared in other format such as thin film[67,74] or magnetic beads[11,59].

2). In this assay, NVP-HRP linking step had lost the proton of amide group that it is strong hydrogen bond site. Thus, the NVP-HRP conjugate procedure should link at the other position.

3). Using other probe such as fluorescent labeled NVP or using biotin labeled NVP in a sandwich ELISA replace enzyme labeled NVP in a direct ELISA.