CHAPTER IV

CONCLUSION

1. Synthesis of HRP labeled theophylline

In order to synthesis HRP labeled theophylline both theophylline and HRP structure have to be modified before conjugation. In this study NHS ester of 7-(3-carboxypropyl)-1,3-dimethylxanthine and aminated HRP were both synthesized in which HRP was labeled at N-7 on the xanthine group of theophylline. The HRP labeled theophylline synthesized containing approximately 11 theophylline molecules per molecule of HRP. The compound exerts HRP activity as well as theophylline property such that it was considered to be the accepted labeled hapten for further use.

2. Preparation of immunogen and antiserum for theophylline

Two immunogens differentiated by the length of side chain at N-7 on xanthine compound were prepared. Immunogen A was synthesized as 7-(3- carboxypropyl)-1,3-dimethylxanthine -BSA conjugate while immunogen B was prepared to be 7- (3- carboxybutyl) -1,3- dimethylxanthine - BSA.

Antibody A and B induced by immunization of immunogen A and B in rabbits exhibited different characterizations in term of titer , capability of antibody in binding to HRP labeled theophylline and specificity. The titer of antibody A and B were 1: 15,000 and 1: 65,000 , respectively. The absorbance related amount of HRP labeled theophylline bound to antibody were 0.967 and 1.953 for antibody A and B , respectively . And the % cross reactivity of antibody A and B were 2.03 and 5.16 , respectively. From this study , it is demonstrated that antibody B showed the higher titer value , the higher capability in binding to HRP labeled theophylline but lower in specificity than antibody A. This finding suppported that reported from Weemen and Schuurs ,1975 ; Hosoda et al., 1981 that using the longer side chain of hapten derivative in the preparation of immunogen would provided the higher capability in binding to HRP labeled theophylline than the shorter side chain as these results above.

Additionally , it was confirmed that antibody induced from immunogen of theophylline conjugated to BSA at the same site as HRP labeled theophylline performed more specificity in binding than those conjugated at the different site. As antibody C was induced from theophylline conjugated to 8-KLH

that different from HRP labeled theophylline. Therefore, in the case of theophylline, bridge heterologous between immunogen and labeled hapten would be used more than site heterologous. However, to get the absolute conclusion, more explanning study should be promoted.

3. Determination of competitive binding properties of HRP labeled theophylline

With the specific antibody induced in this study corporated with the suitable HRP labeled theophylline synthesize, the tendency for developing the immunoassay method for theophylline was demonstrated. In this study it is clearly shown that the competitive reaction between HRP labeled theophylline and theophylline in the sample (within the concentration range of 0 - 40 mg/l) with antibody A or B occured under the appropriate dilution of antibody A of 1 :1,000 and antibody B of 1 : 10,000.

Both antibody A and B can be used in developing enzyme immunoassay method for theophylline. If using antibody C, it is suggested the HRP labeled theophylline should be resynthesized by conjugating the enzyme at C-8 position of theophylline. Therefore, it is overall concluded that

HRP labeled theophylline is successfully prepared for future developing of enzyme immunoassay method of theophylline. The interesting issue that need to be more investigated would be the more explanation on the side - chain heterologous between labeled hapten and immunogen.