CHAPTER V

CONCLUSIONS

1. PheDH from *E. coli* BL 21(DE3) was partially purified by 50-70% saturated ammonium sulfate precipitation and DEAE-Toyopearl with 20.2% yield and 2.6 purification fold.

2. The relative molecular weight of PheDH subunit was estimated to be about 44.5 kDa by SDS polyacrylamide gel electrophoresis.

3. The enzyme was chemically modified with series of group-specific reagents to identify essential amino acid residues. Incubation of the enzyme with 10 mM of *N*-bromosuccinimide (NBS), chloromine T (CT), diethylpyrocarbonate (DEPC) and 2,4,6-trinitrobenzene sulfonic acid (TNBS) which were specific for tryptophan, methionine, histidine and lysine, respectively, led to a complete loss of enzyme activity. The great loss of activity was observed when enzyme was modified with 10 mM PMSF which was specific for serine while *N*-acetylimidazole (NAI), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) and Dithiothreitol (DTT), the modifiers of tyrosine, aspartic or glutamic and cysteine, respectively had little effect on the enzyme activity. Therefore, tryptophan, methionine, histidine, lysine and serine are all likely involved in PheDH activity as the essential residues for enzyme biological functional

4. Covalent immobilization of PheDH via its carboxylic groups gave the highest immobilized activity on silica when compared with other methods and supports.

5. The optimum condition for immobilized enzyme preparation to achieve high immobilized activity was to activate carboxylic groups of enzyme with 10 mM EDC for 6 hours. Silica was activated with 2% (v/v) APTS and 25 units of activated PheDH was added and incubated for 21 hours at 4°C.

6. Under optimal conditions for covalent immobilization of PheDH using carbodiimide, 1.41 U of PheDH was immobilized on 1 g silica with 5.17% of immobilization yield when 25 U of PheDH was applied.

7. Both free and immobilized enzymes showed the same optimum pH and temperature of 9.5 and 40°C, respectively.

8. The immobilized PheDH was stable in the pH range of 5.0-12 whereas the free enzyme showed a narrow range of 5.0-8.5.

9. The thermal stability of free and immobilized enzyme retained its full activity at temperature up to 35°C. The immobilized enzyme lost about half life of its activity at 45°C whereas free enzyme retained only 20% of its activity.

10. Storage stability of the immobilized enzyme form was closely similar to that of the free enzyme when stored at 4°C. At room temperature, the free enzyme lost all its activity within 30 days whereas the immobilized enzyme still retained its activity up to 20%

11. The immobilized PheDH retained 84% residual activity after three repeated use with 58-62.7 % conversion of L-phenylalanine.

12. The application of the immobilized enzyme was extended for amino acid production using their keto acids as substrates. The production yield of L-phenylalanine was 70.9% conversion. Norleucine, leucine, norvaline and methionine were produced with 96.7%, 80.4%, 62.5% and 100% conversion, respectively.