CHAPTER 4

CONCLUSION

4.1 Conclusion

The flow injection-bead injection system for determination of bone ALP in human serum samples was developed. Various ALP isoezymes are released to human serum. Wheat germ lectin can specifically bind to bone ALP (approximately 90%). Wheat germ coated beads were applied to isolate bone ALP form human serum by combining with the FI-BI system followed by the enzyme-substrate reaction for detection. The aim here is to develop and demonstrate that the simple flow injection-bead injection system can be used to perform online assay and screen for patients with bone diseases. The proposed system offers good precision, short analysis time (about 30 minutes per analysis), the ease of operation, reduce the direct contact between the operator with blood sample and lower cost than conventional system as shown in Table 4.1. From the Table 4.1, a high cost of the FI-BI assay can be reduced by coupling wheat germ lectin on beads by self. The increase of beads volume led to increase sensitivity due to increase capacity. A high sample volume can be decreased by reducing distance between sample injection-valve to detector led to decrease dispersion. The FI-BI system correlated with commercial ELISA kit at significantly level 0.001 and can better differentiate bone diseases form normal persons as compared to the ELISA kit. Therefore, this developed method has high potential of being used to screen and monitor for bone disorders.

Table 4.1 The comparison of the FI-BI assay with commercial ELISA kit

Characteristics	FI-BI	ELISA*
Method	Wheat germ lectin-capture	Immuno-capture
Analyte	Bone ALP	Bone ALP
Sample	Serum	Serum
Sample volume	200 μl	20 μl
Sensitivity	6.3 U/L	0.7 U/L
Within –run precision	6 %RSD	4-6 %RSD
Between-run precision	5 %RSD	5-8 %RSD
Operation time	25 min	> 3.5 hours
Sample size	1 sample/run	40 samples/plate
Cross reactivity with liver ALP	3-7 %	3-8 %
Estimated cost	400 baht/run	550 baht/run

* The results were obtained from manufacture.

4.2 Further works

The following works should be done in the future to improve the proposed system.

1. Higher degree of automation using sequential injection analysis (SIA) for auto-injection of samples and substrate

2. Investigation for estimating the cut-off value using more samples

THE RELEVANCE OF THE RESEARCH WORH TO THAILAND

To diagnose bone diseases such as osteoporosis, bone mineral density (BMD) is normally measured by using a bone scan instrument (Dual-energy x-ray absorptiometry, DEXA) which is very expensive instrument. Bone turnover is generally so slow that it may take up to 2 years in most circumstances to be certain that there is any change in BMD. Therefore, the treatment monitoring is difficult. Bone ALP is one of bone marker that has been used to monitor the bone response to the medical treatment. Several methods for bone ALP assay are tedious, require long analysis time, involve complex operation and some methods are expensive such as HPLC and immunoassay. This work aims to develop the bone ALP assay system that can reduce those problems by using wheat germ coated micro-beads with a simple FI-BI system. The advantages include the ease of operation, good precision, shorter analysis time and lower cost. Each analysis can be completed in 30 min. Its relatively lower cost of instrumentation and analysis supports the Thailand's goal of having a high quality and affordable health care for everyone.