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A Brief Review on Fast Protein Liquid Chromatography- FPLC

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ABSTRACT:

This review shows an overview about the Fast Protein Liquid Chromatography which is an embodiment of high performance liquid chromatography. This technique has an advantage for the higher resolution of separation for particle of small average diameter in stationary phase. These methods have more loading capacity, better biocompatible and high separation of proteins. AKTA FPLC is recently used for purification of protein. This method is also applicable for some other different kind of biological samples also.

KEY WORDS: Separation, FPLC, stationary phase, purification, protein

INTRODUCTION

FPLC is a Fast Protein Liquid Chromatography which is an embodiment of high performance liquid chromatography. Chromatography with high resolution is widely depended upon the packing of the column with the small particle size. And they have straight relationship between the parameter of theoretical plate height, particle size and H with the column for reducing the broadening of peak. Column packing utilize an average particle size in HPLC, which make it limited to low loading of sample and required high pressure along with organic solvents. [1-3]

In 1982, Fast Protein Liquid Chromatography is developed for better biocompatible separation of biopolymers by high resolution, such as proteins and Pharmacia LKB.[4] The automated liquid chromatography is widely use now a day also called as AKTA Fast Protein Liquid Chromatography is produced by Amersham Pharmacia Biotech, Sweden for the purification of proteins.[4] Different set of chromatography mode is given by FPLC like ion exchange [5-7], gel filtration [8], reverse phase [9] along with hydrophobic interaction[8], by using the particles having an diameters of average size for separation. In FPLC the sample loading is higher than that of HPLC.

MATERIALS

"In these various types of material is used:-

- Various instruments are used for FPLC such as AKTA FPLC explorer, Amersham Pharmacia Biotech AB, Sweden.
- Buffer A: 10mM Tris-HCl, pH 7.0, Filer (0.22 µm filter) and Degas
- Buffer B: 10 mM Tris–HCl, pH 7.0, 1 M NaCl. Filter (0.22 µm filter) and degas"
- Buffer C: 50 mM Tris–HCl, pH 7.0, 100 mM KCl

INSTRUMENTATION

Composition of FPLC system

1. Program controller
2. Two pumps
3. Injection loop
4. Column
5. Mixer
6. Injection Valve
7. An Ultraviolet monitor
8. Fraction Collector
9. Recorder

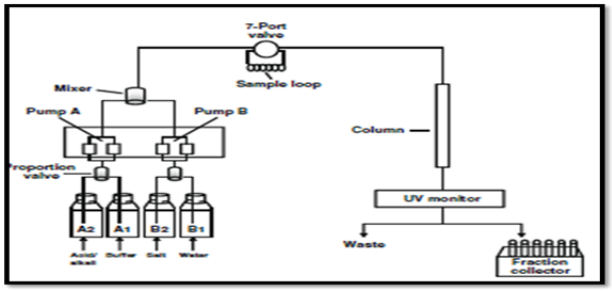

Figure 1 Schematic Outliene of AKTA Explorer FPLC System

1. Program controller: - this is an important part in chromatography systems. Program control is generally control the process and fraction collectors, adjust the separation parameters, determine data and also gives the print information from the chromatogram.
2. Pumps:- Various Pumps are generally used in these chromatography such as, 2 cylinder piston pumps, peristaltic pump etc. pumps are generally act as an accurately on high or low rates in their whole pressure range.
3. Mixer: - These are generally used to mix the buffers and run the gradient flow of buffer from low to high concentration.
4. Injection loop:- the volume of the injection loop from milliliters to 50 ml
5. Injection valve:-injection valve is connecting to the mixer and sample loop to the column. This valve is generally used to inject the sample into the column. Sample can be injected in range of 1 – 150ml by using syringes of 10, 50, 150 ml
6. Column: - Columns are generally made up of glass. Diameter is generally 5-50 mm and height is 5 cm to 1 meter. Generally column is semi sealed in bottom with filter which allows the precipitation of loaded samples.
7. Ultraviolet monitor:- these part is determine the pH UV absorption and conductivity of the sample interest
8. Fraction Collector: - It is generally a rotating rack that can be filled with test tubes.
9. Recorder: - Generally recorder is connected to the flow cell. On older, system was very simple and chart recorder but in modern systems, computer with hardware interface and display is also used.[10]

WORKING

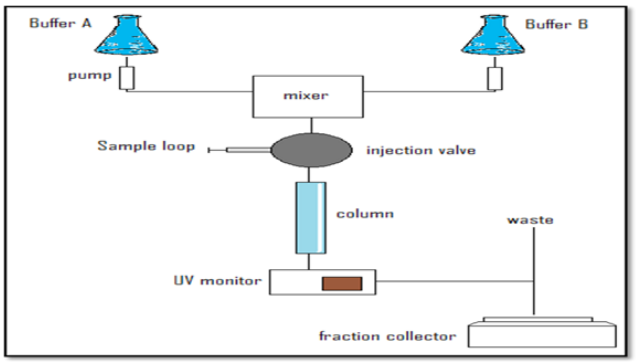

Figure 2 Procedure of FPLC

1. The constant flow of buffers/ solvents is very important in Fast Protein Liquid Chromatography.
2. The solvent [Buffer A and Buffer B] is filled into reservoir which is connected to pump

- It can allow the solvent to pass to the mixer.
- In this buffers used in this process is mixed

3. Then elution of solution is done on the basis of different buffer concentration. For this the concentration of buffer B solvent is gradually increases from 0%-98%.

Injection valve can inject the sample

4. It can be carried out in column by the flow of solvent from the mixers
5. In the column separation of sample is done on the basis of affinity and solubility towards the stationary phase with constant flow of buffer in the system.
6. After that eluted samples are collected by using fraction collector and the data are observed by monitor. [10]

ADVANTAGES

- It can be used for separating different proteins from urine, plasma and cerebrospinal fluid.
- Also used for separating Peptides and Polynucleotide.
- Plasmid DNA and RNA purification.
- Separation of Lipoprotein.
- Estimation of nitrogen containing compounds in beer.
- Useful for determination of protein structure and sequence of peptides.

- Separation of complex protein in simple liquid phase.
- Pancreatitis Analysing: some proteins which can causes disease can be determined by using FPLC in pancreatic juice.
- Plasma Profiling: In FPLC the proteins can be profiled by anion exchange column. It can determine what types of changes occur in the body of patient.

DISADVANTAGES

- Glass column is required.
- High pressure is not suitable for this technique.
- It should not support HPLC columns.
- Heat sensitive protein cannot be easy to determine.[10]

APPLICATION OF FPLC

- Ion Exchange Chromatography
- Affinity Chromatography
- Reverse-Flow chromatography
- Gel Filtration Chromatography
- Hydrophobic Interaction Chromatography"
- Hydroxyapatite chromatography[10]

CONCLUSION

In this review we can conclude about the FPLC approach which has great ability for separation of biopolymers like proteins and some biological samples. These methods have an automated liquid chromatography, having better biocompatible for the separation of samples.

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REFERENCES

1. Liu, H., Finch, J. W., Lavallee, M. J., Collamati, R. A., Benevides, C. C., Gebler J.C: Effects of column length, particle size, gradient length and flow rate on peak capacity of nano-scale liquid chromatography for peptide separations: Journal of Chromatography A. (2007); 1: 30–36.

2. Sheehan D: Chromatography in Physical Biochemistry. Principles and Applications, 2nd Edition, Wiley-Blackwell: Chichester, UK, 2009: 11–51.

3. Cabrera, K: Applications of silica-based monolithic HPLC columns: Journal of Separation Science. (2004); 27: 843–852.

4. Tangvarasittichai, S., Tangvarasittichai, O., Jermnim, N: Comparison of fast protein liquid chromatography (FPLC) with HPLC, electrophoresis and microcolumn chromatography techniques for the diagnosis of b-thalassaemia: Indian Journal of Medical Research. (2009); 129: 242–248.

5. Glanc, P., Galbas, M., Dullin, P., Szalata, M., & Slomski, R: The application of FPLC chromatography (Mono Q) for the purification of wheat RNA polymerase II and III: Cellular and Molecular Biology Letters. (2003). 8: 825–830.

6. Plank, J., Andres, P. R., Krause, I., Winter, C: Gram scale separation of casein proteins from whole casein on a Source 30Q anion-exchange resin column utilizing fast protein liquid chromatography (FPLC): Protein Expression and Purification. (2008); 60(2): 176–181.

7. Boesze-Battaglia, K., Kong, F., Lamba, O. P., Stefano, F. P.,Williams, D. S: Purification and Light-Dependent Phosphorylation of a Candidate Fusion Protein, the Photoreceptor Cell Peripherin/rds: Biochemistry. (1997); 36(22): 6835–6846.

8. Riaz, M., Perveen, R., Javed, M. R., Nadeem, H., Rashid, M. H: Kinetic and thermodynamic properties of novel glucoamylase from Humicola sp: Enzyme and Microbial Technology. (2007); 41(5): 558–564.

9. Miyazaki, O., Fukamachi, I., Mori, A., Hashimoto, H., Kawashiri, M., Nohara, A., Kobayashi, J: Formation of preβ1-HDL during lipolysis of triglyceride-rich lipoprotein: Biochemical and Biophysical Research Communications. (2009); 379(1): 55–59.

10. Fast protein liquid chromatography. Retrived from https://fplc.weebly.com/index.html