www.ijpbs.net

INTERNATIONAL JOURNAL
OF PHARMA AND BIO SCIENCES

Internationally indexed journal

Indexed in Chemical Abstract Services (USA), Index coppernicus, Ulrichs Directory of Periodicals, Google scholar, CABI ,DOAJ , PSOAR, EBSCO , Open J gate , Proquest , SCOPUS , EMBASE ,etc.

Rapid and Easy Publishing

The "International Journal of Pharma and Bio Sciences" (IJPBS) is an international journal in English published quarterly. The aim of IJPBS is to publish peer reviewed research and review articles rapidly without delay in the developing field of pharmaceutical and biological sciences

Pharmaceutical Sciences

- Pharmaceutics
- Novel drug delivery system
- Nanotechnology
- Pharmacology
- Pharmacognosy
- Analytical chemistry
- Pharmacy practice
- Pharmacogenomics
- Polymer sciences
- Biomaterial sciences
- Medicinal chemistry
- Natural chemistry
- Biotechnology
- Pharmacoinformatics
- Biopharmaceutics

Biological Sciences

- Biochemistry
- Biotechnology
- Bioinformatics
- Cell biology
- Microbiology
- Molecular biology
- Neurobiology
- Cytology
- Pathology
- Immunobiology

Indexed in Elsevier Bibliographic Database (Scopus and EMBASE)
SCImago Journal Rank 0.129
Impact factor 0.47*

*Instruction to Authors visit **www.ijpbs.net**

For any Queries, visit "contact" of www.ijpbs.net

Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 875 - 884

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

FUZZY APPROACH FOR THE ESTIMATION OF THE AMOUNT OF PURIFIED PROTEIN IMMUNOGLOBULIN G FROM THE PROTEIN A AFFINITY COLUMN

ABDUL MANNAN[1*] AND PON.SRIGAYATRI[2]

[1]Applied Mechanics Department, Motilal Nehru National Institute of Technology, Allahabad, 211004, India
[2]Talwar Research Foundation, E-8, Neb Sarai, New Delhi, India, 110068,

ABSTRACT

In the process of protein purification, the amount of proteins isolated with the help of commercial protein purification processes remains uncertain and vague. The present paper proposed a set of fuzzy rule system based on Fuzzy Expert System (FES) which predicts the amount of purified proteins based upon the flow rate of the protein in column, pH and binding capacity of resin to desired protein present in column. The potential benefit of this fuzzy system is to develop a computational model which helps the scientific community to determine the amount of purified protein before starting the process of purification and saving time and related procedural works.

KEYWORDS: Fuzzy Expert System, Protein Purification, Affinity column chromatography, Flow rate, Binding capacity.

ABDUL MANNAN
Applied Mechanics Department, Motilal Nehru National Institute of Technology, Allahabad, 211004, India

Corresponding author

INTRODUCTION

The need of protein purification is to characterize the structure, functionality, and interaction mechanisms of the protein with other molecules. Protein purification is a primary step to be followed and it is vital step in biology for the contribution in vaccine production, antibody purification and purification of various biochemical products. Depending upon charge, size, hydrophobicity and biorecogonition, the protein is purified through various methods like ion exchange, mass exclusion, gel filtration, hydrophobic interaction and affinity column[1]. During protein purification, several levels of uncertainty and imprecision are involved about the exact amount of protein purified. In this paper we presented a FES for estimating the amount of purified protein. For development of such system, we considered the purification of IgG protein through affinity column chromatography using Protein A resin. Affinity chromatography separates proteins on the basis of a reversible interaction between single protein (or group of proteins) and a specific ligand coupled or conjugated to a chromatographic matrix like sepharose and Agarose. Affinity chromatography using Protein A can be used to isolate monoclonal and polyclonal IgG from ascities, serum and tissue culture and bioreactor supernatant[2]. In this chromatography flow of supernatant containing desired IgG protein should mange and it set with a specific flow rate which runs through the resin present in the column. The flow rate is managed in such a way that the supernatant (protein) gets enough time to bind with the resin. Table 1 shows the Influence of flow rates on binding of protein with resin in the column[3]. During this binding process of IgG to Protein A, pH is an important parameter which can lead to effective binding. The binding capacity of IgG is more when the sample is loaded onto the column with a buffer at a neutral pH and physiological ionic strength (i.e., PBS, pH 7.4). Finally the bound proteins are eluted with elution buffer having low pH[4].

Table 1
Influence of flow rates on binding of protein with resin[3].

S.No	Flow Rate	Average of Bound Protein with corresponding flow rate (mg protein/ml resin)
1.	0.2ml/min	35.8
2.	1ml/min	27.4
3.	4ml/min	19.5

Protein A is a cell wall component produced by several strains of *Staphylococcus aureus* which consists of a single polypeptide chain of molecular weight 42 kDa and contains little or no carbohydrates[5,6]. Protein A binds specifically to the Fc region of immunoglobulin molecules, especially IgG. Immobilized Protein A through different resin has been used extensively for the isolation of IgG from several species of mammals[7]. Table 2 presents the binding capacity and flow rate of several subclass of IgG with different types of resin[8].

Table 2
The binding capacity and the flow rate for different types of resin.

S.No	resin	Binding capacity	Maximum operating flow	Comments
1	HiTrap Protein G HP	Human IgG, > 25 mg/column Human IgG, >125 mg/column	4 ml/min (1 ml column) 20 ml/min (5 ml column)	Purification of IgG, fragments and subclasses, including human IgG3.Strong affinity for monoclonal mouse IgG1 and rat IgG.
2	MAbTrap Kit	Human IgG, > 25 mg/column	4 ml/min	Purification of IgG, fragments and subclasses, including human IgG3. Strong affinity for monoclonal mouse IgG1 and rat IgG.
3	Protein G Sepharose 4 Fast Flow	Human IgG, > 20 mg/ml medium Cow IgG, 23 mg/ml medium Goat IgG, 19 mg/ml medium Guinea pig IgG, 17 mg/ml medium Mouse IgG, 10 mg/ml medium Rat IgG, 7 mg/ml medium	400 cm/h	

Fuzzy Expert Systems

According to natural biological, non-linear thinking in the computerized world, intelligent techniques are the most advanced modeling techniques are similar to human thinking and judgment which can evaluate and decide based on an inference process. Some of the widely used intelligent techniques are fuzzy techniques, neural network techniques, genetic algorithms and knowledge based systems (also known also as expert systems)[9]. Zadeh et al. (1965)[10] originated the "fuzzy logic" or "fuzzy set theory" which is based on the nature of fuzzy human thinking. Fuzzy logic provides reasoning methods capable of making approximate inferences[11] and deals with the problems those have fuzziness or vagueness. The degree of membership is in a given set that may be anywhere in the range of zero (completely not in the set) to one (completely in the set) for a particular object in a fuzzy set theory[12]. FES is based on the fuzzy set theory. Mamdani method is widely used for the development of FES. Mamdani applied a set of fuzzy rules supplied by experienced human operators. Following steps were adopted for developing the Mamdani procedure[9,10]:

1. Fuzzification of the input and output variables. The first step is to take the crisp input and determine the degree to which this input belongs to each of the appropriate fuzzy set and thus fuzzy membership values is are found out.
2. The obtained fuzzy values are then processed in fuzzy inference mechanism where the Fuzzy rule evaluation takes place on the basis of expert knowledge.
3. Aggregation of the rule output.
4. Defuzzification to generate the crisp output data.

The aim of this paper is to describe an intelligent procedure based on fuzzy techniques that could be used to estimate the amount of purified protein from Protein A resin column chromatography. This estimation approach requires the definition of a set of judiciously chosen intelligent fuzzy rules concerning the binding capacity of protein to resin, pH and flow rate of solution containing desired protein.

METHODOLOGY

FES for estimation of purified IgG by Protein A resin column chromatography has three input variables viz. flow rate, pH of the solution

containing IgG protein and binding capacity of IgG to Protein A resin and one output variable consist of percentage of purified protein IgG. For designing FES, linguistic controlling strategy is applied which depends on the expert human knowledge[13].

(i) Input Variables

Three input variable with ranges required for fuzzy implementation are given as follows:

Flow Rate: For maximum binding capacity of IgG with Protein A, lower flow rates are recommended those yield higher percentage of purified protein. Input variable flow rate (ml / min) was described by a set of five linguistic fuzzy values – very slow, slow, medium, fast and very fast as given in table 3 and each fuzzy rule is defined through a gauss membership function depicted in figure 1.

Table 3

Input variable- flow rate in ml / min with five linguistic fuzzy values.

Flow Rate in ml / min	
Very Slow	< 0.5
Slow	0.5 to1
Medium	1 to 2
Fast	2 to 3
Very Fast	> 3

Membership function graph

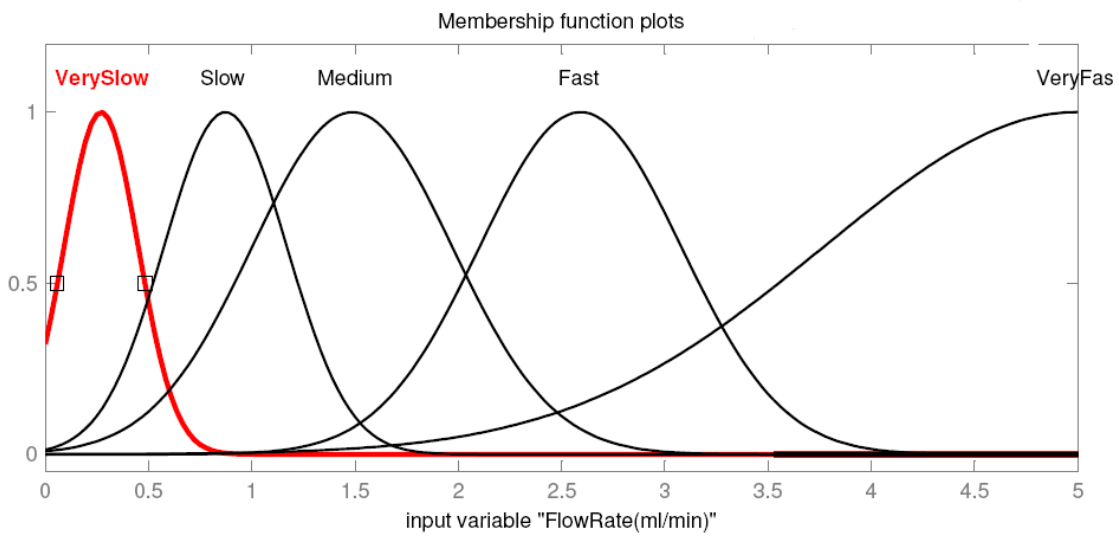

Figure 1

Membership function graph for the input variable "Flow Rate" with five possible fuzzy values – very slow, slow, medium, fast, very fast.

pH

For the proper binding of IgG to the resin, neutral pH is recommended. Input variable pH was described by a set of three linguistic fuzzy values – low pH, Neutral pH and High pH is shown in table 4 and each fuzzy value is defined through gauss a membership function which is given in figure 2.

Table 4
Input variable- pH of supernatant with three linguistic fuzzy values.

pH of Supernatant	
Low pH	1 to 6.9
Neutral pH	7.0to 7.9
High pH	8.0 to 12

Membership function graph

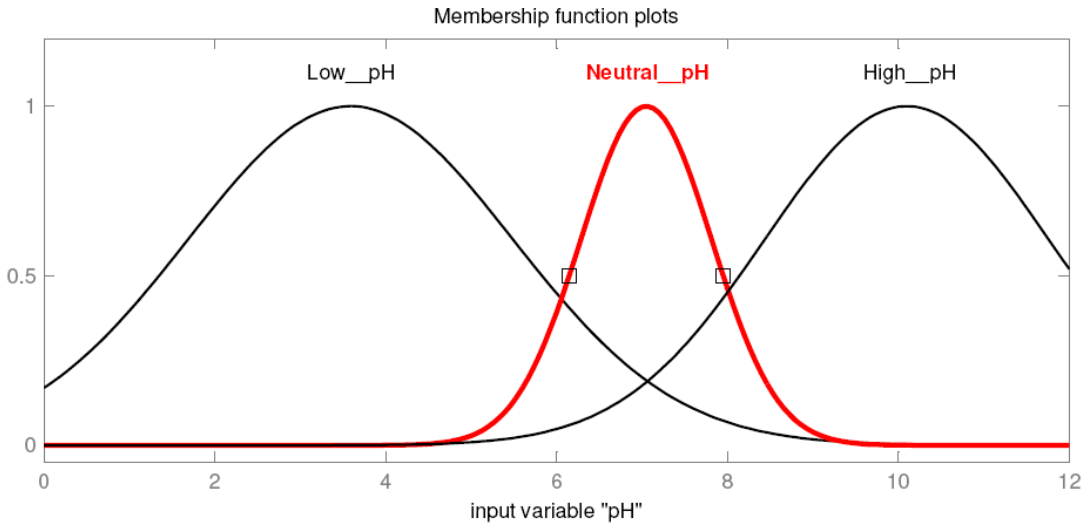

Figure 2
Membership function graph for the input variable "pH" with three possible fuzzy values – low pH, neutral pH and high pH.

Binding Capacity of IgG to Protein A resin

The Protein A molecule contains four high affinity (Ka = 10^8/M) binding sites capable of interacting with the Fc region of Immunoglobulin[14]. The binding capacity varies among different resins obtained from various manufactures (Table 2) and the average binding capacity of IgG to protein A resin was taken in consideration[8]. Input variable binding capacity was described by a set of four linguistic fuzzy values – less, moderate, high and very high given in table 5 and each fuzzy value is defined through a gauss membership function which is depicted in figure 3.

Table 5
Input variable- Binding capacity of IgG to protein A resin in mg / ml with four linguistic fuzzy values

Binding Capacity of IgG to Protein A resin (mg/ml)	
Less	<5
Moderate	5 to 10
High	10 to 20
Very High	> 20

Membership function graph

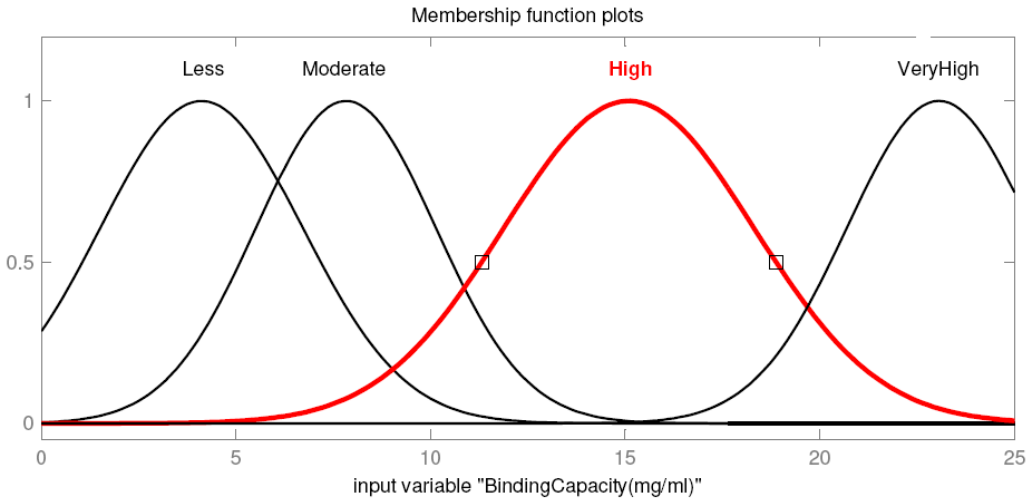

Figure 3
Membership function graph for the input variable "Binding Capacity" with four possible fuzzy values – less, moderate, high, very high.

(ii) Output Variables

The output variable is the estimated purified IgG, which is given as the percentage of purified protein (IgG), described by a set of four linguistic fuzzy value – Very Low, Low, Moderate, High and Very High as given in table 6 and its fuzzy values are defined through a gauss membership function which is depicted in figure 4.

Table 6
Output variable – amount of purified protein (IgG) in percentage with five linguistic fuzzy values

Percentage Amount of Purified Protein (IgG).	
Very Low	< 5
Low	5 to 20
Moderate	15 to 40
High	30 to 50
Very High	40 to 80

Membership function graph

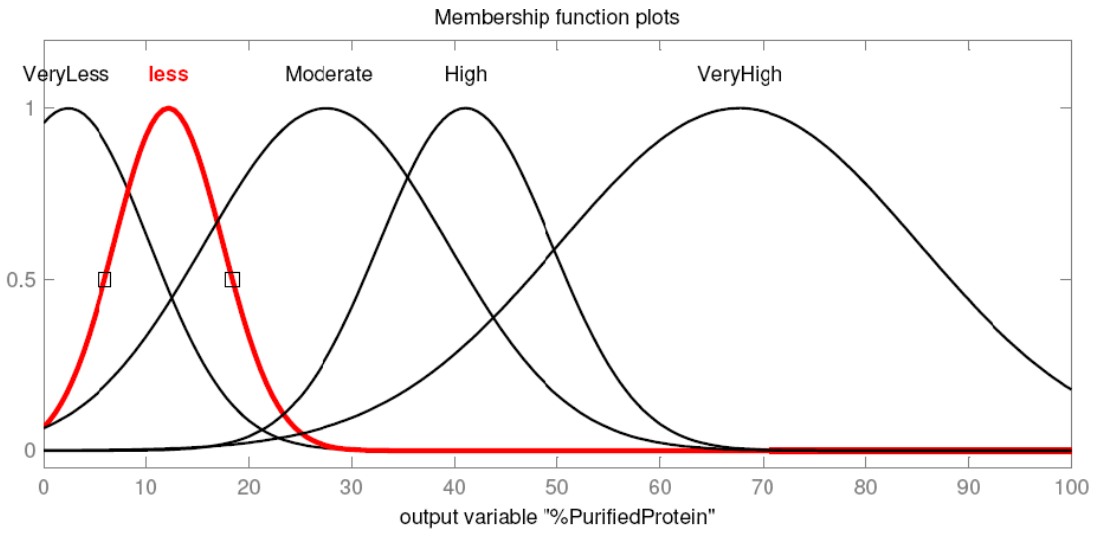

Figure 4

Membership function graph for the output variable "% Purified Protein" with 5 possible fuzzy values – very less, less, moderate, high, very high.

The general structure of developed FES which includes 3 input variables and 1 output variable for the estimation of purified protein IgG from Protein A Affinity Column chromatography is depicted in figure 5. This system was designed and investigated using MATLAB software tools.

General structure of the FES

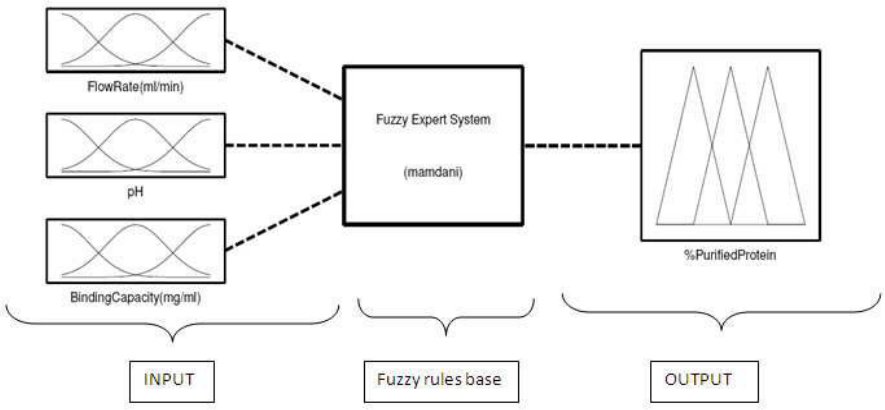

Figure 5

General structure of the FES for the estimation of purified protein IgG from Protein A Affinity Column which includes the fuzzification of input, evaluation of fuzzy input through fuzzy base rules, aggregating the output and finally defuzzification to get the crisp output.

(iii) Rule of Fuzzy System

The fuzzy rules were designed on the basis of individual concepts as per the variability of the data and fuzzy rule normally collected with the help of expert knowledge. In the fuzzy rule input variables were connected by the connecter AND, and rule based on "If – then" rule[11]. According to the input and output variables,

total 60 rules were possible in the system. Some of fuzzy rule are given below:

Rule 1: If (Flow Rate (ml/min) is Very Slow) and (pH is Low pH) and (Binding Capacity (mg/ml) is Less) then (%Purified Protein is Very Less)

Rule 2: If (Flow Rate (ml/min) is Very Slow) and (pH is Low pH) and (Binding Capacity (mg/ml) is Moderate) then (%Purified Protein is Less)

…….

Rule 6: If (Flow Rate (ml/min) is Very Slow) and (pH is Neutral pH) and (Binding Capacity (mg/ml) is High) then (%Purified Protein is High)

……

Rule 48: If (Flow Rate (ml/min) is Very Fast) and (pH is Low pH) and (Binding Capacity (mg/ml) is Very High) then (%Purified Protein is Less)

Rule 53: If (Flow Rate (ml/min) is Very Fast) and (pH is High pH) and (Binding Capacity (mg/ml) is Moderate) then (% Purified Protein is Very Less)

……..

Rule 60: If (Flow Rate (ml/min) is Very Fast) and (pH is High pH) and (Binding Capacity (mg/ml) is Very High) then (%Purified Protein is Less).

(iv) Defuzzification

Mamdani approach is used for the output mechanism. For the determination of the percentage of the purified protein (IgG) on the basis of linguistic input variable such as flow rate, pH of the solution containing IgG protein and binding capacity of IgG to Protein A resin, a rule base is evaluated and depending upon that, fuzzy value for estimated purified IgG is evaluated. The estimated value for purified IgG is a fuzzy value. This fuzzy value is converted into crisp value by the process called defuzzification. Defuzzification process was performed to aggregate the output fuzzy set which provides a single crisp value. In the defuzzification the exact output is obtained with “centroid” method according to validity degree.

RESULTS

In the combination of the input-output variables and fuzzy rules mentioned above, the results were presented after defuzzification and simulation surface of the above described FES which describes the direct relationship among the considered input and output parameters. The Surface Viewer showed the dependency of the outputs for any two of the three inputs that is, for the whole system, it designs and create an output surface map. So in order to see the whole output system, the surface viewer produce the entire output map by combining and comparing with any two input data sets. In figure F surface viewers displays the dependency of output on different combination of two inputs like flow rate and pH (Fig. 6.1), Flow rate and binding capacity (Fig. 6.2), and pH and Binding capacity (Fig. 6.3).

Surface Viewer

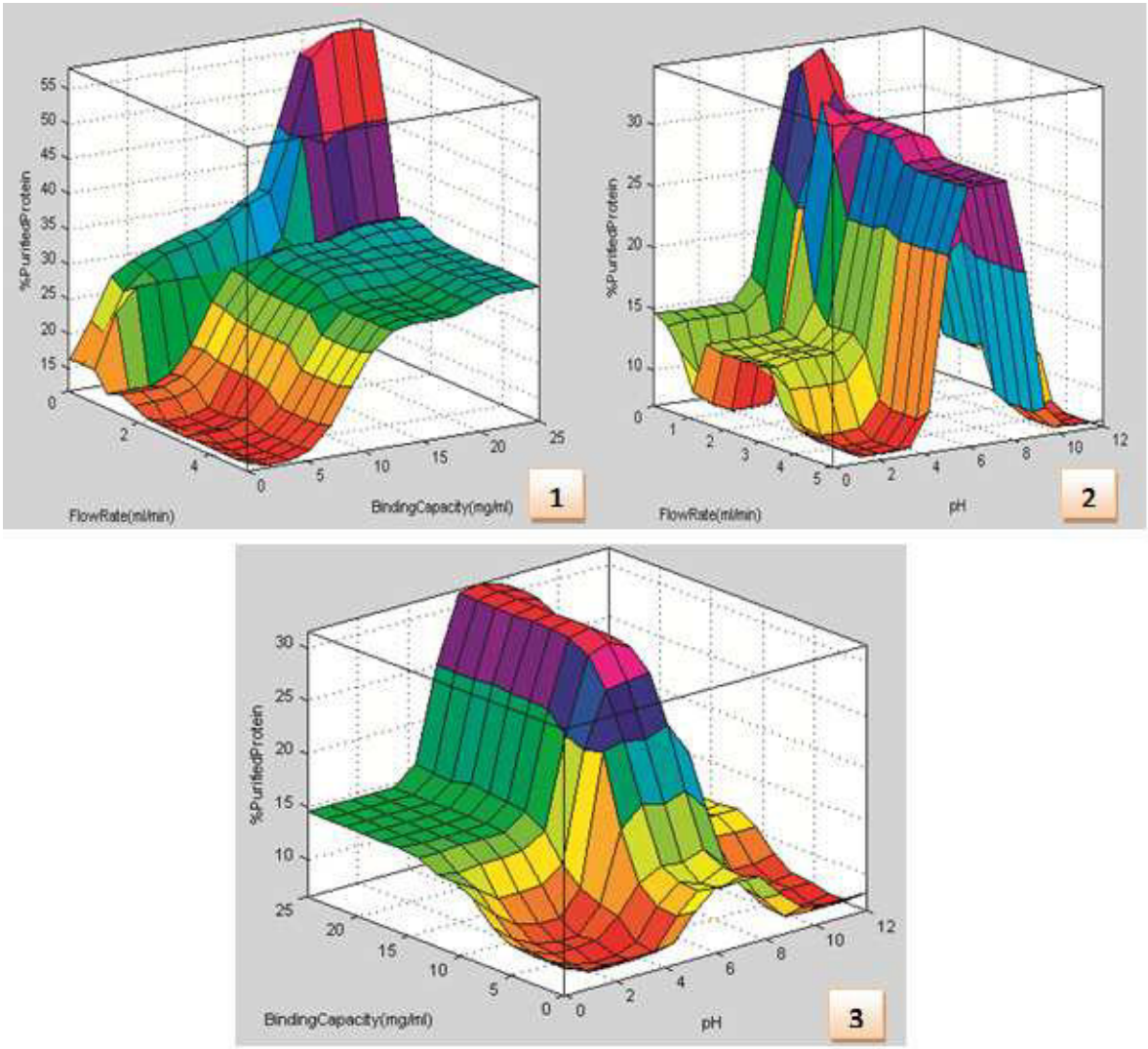

Figure 6.1 ***3D Surface Viewer for % Purified Protein with Respect to Flow Rate and pH.***
Figure 6.2 ***3D Surface Viewer for % Purified Protein with Respect to Flow Rate and Binding Capacity.***
Figure 6.3 ***3D Surface Viewer for % Purified Protein with Respect to Binding Capacity and pH.***

DISCUSSION

The purified protein is estimated by different methods and none of those are 100% accurate. Lots of incertitude and imprecision are involved regarding the amount of protein estimated by different methods. Now a day's computer based fuzzy logic techniques are becoming powerful enough to surpass expert choice. The present research article confirms that the fuzzy inference model is competent enough to capture expert knowledge and experimental lab observation in scientific way. The aim of this study is to propose a FES for the estimation of purified protein IgG from protein A column on the basis of flow rate of the protein in column, pH and binding capacity of resin to desired protein. The fuzzy rules link the flow rate of protein with pH and binding capacity of resin to estimate the amount of protein purified from protein A column. The defined fuzzy logic rules result in reasonable agreement with the results of various classic protein quantification methods. Though it needs further calibration and validation on more inputs such as ionic strength, sample concentration and sample

origin from different species, this procedure can be easily integrated in quantification procedures to automatically estimate the amount of protein purified from the column. Future results are expected for better optimization of fuzzy rule by analyzing different input variables to improve system performance.

ACKNOWLEDGMENT

We are grateful for discussion with Dr. Pankaj Shrivastava, Mathematics Department, MNNIT, Allahabad, Prof. G.P. Talwar and Dr. J. C. Gupta Talwar Research Foundation, New Delhi. We thank Mr. Sagar Udaseen, NIT Rourkela for their guidance to prepare the manuscript.

REFERENCES

1. R. K. Scopes,Ed. Protein purification: principles and practice, 3rd Edn, Springer:1-14, (1994).
2. S. M. Andrew and A. J. Titus. Antibodies as cell biological tools. In: Jennifer Lippincott-Schwartz (eds.), *Current protocols in cell biology*, John wiley and sons. Inc, New York, 2000,pp.1-12.
3. Pall lifesciences. Acrosep protein A columns. Available at: http://www.pall.co.in/pdfs/Laboratory/Protein_A_PII.pdf, 2008.
4. R. C. Duhamel, H. Peter, P. H. Schura, et al, pH gradient elution of human IgG1, IgG2 and IgG4 from proteinA-Sepharose. Journal of Immunological Methods, 31(3-4): 211-217,(1979).
5. L. Bjork, B. A. Petersson J. Sjoquistet, Some physicochemical properties of protein A from *Staphylococcus aureus*. Eur. J. Biochem, 29(3): 579-84,(1972).
6. J. Sjoquist, B. Meloun, H. Hjelm, Protein A isolated from Staphylococcus aureus after digestion with lysostaphin. Eur. J. Biochem, 29(3): 572-578, (1972).
7. H. Hjelm, J. Sjodahl, J. Sjoquist, Immunologically active and structurally similar fragments of protein A from Staphylococcus aureus. Eur. J Biochem, 57(2): 395-403, (1975).
8. Amersham Pharmacia Biotech. Affinity chromatograph: Purification and methods. Available at:http://wolfson.huji.ac.il/purification/pdf/affinity/pharmacia-affinitymanual.pdf, pp.28-37, (2001).
9. V. Balanica, I. Dumitrache, M. Caramihai, et.al., Evaluation of breast cancer risk by using fuzzy logic. U.P.B. Sci. Bull, 73(1): 53-64, (2011).
10. L. A. Zadeh, Fuzzy sets. Information and control, 8(3): 338-353,(1965).
11. R.E. Bellman, L. A. Zadeh, Decision making in a Fuzzy Environment. Management Science, 17(4): 141-164, (1970).
12. J.wesley Hines, Fuzzy and Neural Approaches in Engineering: Matlab supplement. In: Lefteri H. Tsoukalas, Robert E. Uhrig, Lotfi A. Zadeh (eds.), *Fuzzy and Neural Approaches in Engineering*, John Wiley & sons, New York, 1997,pp16-18.
13. N. Allahverdi, G. A. Altan, Fuzzy Expert System Design to Monitor Patient's Condition during Heart Surgery, CompSysTech '11 Proceedings of the 12th International Conference on Computer Systems and Technologies, Vienna: 335-340, (2011).
14. G. Kronvall, R. C. Williams, Differences in anti-protein A activity among IgG subgroups. J. Immunol, 103(4): 828-33, (1969)