

Application and development of proteomics in biopharmaceutical industry

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Abstract: *The biopharma and diagnosis industry are engaged in an ongoing improvement toward new drug-target interaction or new markers establishing a better diagnosis, prognosis, or risk assessment. Proteomics is the analytical chemistry of proteins on a large scale and every inch of this science plays its role in understanding the possible performances of proteomics. Drug discovery technology can pose limitations in proteomics analysis and the severe problems related to protein synthesis. Identification of several diagnostic markers, candidate vaccine production, pathology of a disease, altered protein expression in the response of different signals, and understanding of protein pathways in different infectious diseases can be done based on various research settings are utilized by proteomics-based technologies. Moreover, the proteome is highly dynamic because of complex regulatory systems that control the expression levels of proteins. Since the last few years of industrial evolution, the development of proteomics in the pharmaceutical industry has showcased the production within academia in comparison with other industries. Starting from the initial success and rapid disappointment in global protein expression profiling, research in the pharmaceutical area witnessed the largest impact while relating to more focused applications such as the interaction between proteins and drugs. In recent days, proteomics technologies have been integrated with many areas of pharmaceutical R&D, ranging from the analysis of therapeutic proteins to the monitoring of clinical trials. Here, we have studied various applications and analyses of proteomics, placing it in a historical context as well as reviewing the current status in light of the contributions to the biopharmaceutical industry and as well as in the research and development process.*

Key Words: *Proteomics, Pharmaceutical biotechnology, Biopharmaceutics, Research and development, Protein expression, Industrial evolution.*

1. INTRODUCTION:

In analytical chemistry, the diverse structural and physicochemical properties of proteins are difficult to be understood properly, so the study of proteins is considered to be the most difficult and complex one. However, vigorous development and improvement in the field of proteomics have been achieved [1]. The study of proteomics deals with the treatment of disease by any biomolecules or drugs derived from the plant peptides or phytohormones. It is required to explore deeper and deeper in a specific proteome pressured researcher to triumph over the boundaries of pre-fractionation techniques, the robustness of mass spectrometers, and the scope of bioinformatics software because of the complexity of the pattern to be analysed imposed exclusive techniques for success [2]. In any cellular or biological function, proteins play a pivotal role in the functioning of the organs and gene expression. Proteomics study can be used as the most important tool to evaluate the protein expression level. In medical diagnosis and clinical research to assess the biological and cellular activities, proteomic analysis is very much essential to isolate different diseases. The term proteome was first coined in 1994 which is the main tool to study proteomics in several fields of health diagnostics purpose. Proteomics is not only a branch of research and study; it also has a greater impact in the industrial area over the last few years. Quality control and validation of the food products can be handled using the proteomics method and as well as identification of the drug targets in the drug discovery pipeline to find a proper target-based ligand molecule against human disease as the preliminary diagnostic step. Finding a target-based drug is not an easy job, it is a very complex and time-consuming procedure for which a drug should satisfy various criteria. Since proteomics can also involve protein-protein interaction in the fundamental cellular system, it can be a valuable method in the field of drug discovery [3]. In a specific biological process, the identification and detection of a particular protein can be determined by quantitative proteomics which is the most frequently studied method, by quantifying the protein in several situations which shows significant differences in protein abundance among defined proteomes [4]. Different types of quantitative approaches of proteome analysis have been deployed nowadays by considering several bottlenecks, challenges, advantages, and disadvantages [5]. Using liquid chromatography and

tandem mass spectrometry analysis, the particular protein can be detected and quantified as in the case of label-free quantification. Mass spectrometry frequencies and signal intensities can be used for spectral counting which is attributed to peptides involving in a specific protein are considered. Another important field in proteomics study is protein post-translational modification (PTM) which is dependent on the aim of the study. To obtain a successful result, a sample should be prepared given qualitative and quantitative approaches with the PTM method [6]. Few PTM methods are including acetylation, glycosylation, methylation, oxidation, ubiquitylation, and likewise. By using predictive tools, the data analysed by PTM are acquired and made available to the researchers to detect modification areas and databases having information about modified proteins [7]. In the pharmaceutical industry, proteomics plays a pivotal role to produce targeted drugs to reduce the disease progression human body. The most significant biopharmaceutical product is used as a vaccine consists of antigenic protein which is the major studied portion expressed in plants [8]. The edible vaccine can be produced with the help of plant-based food products like tomato, carrot, potatoes, and lettuce leaves which would be a major cost-effective use of antigenic protein in vaccine production [9]. One of the major applications of proteomics can be applied in human insulin development for the treatment of type I diabetes mellites by targeting the molecule to seed oil bodies. On the other hand, monoclonal antibodies are in development in the treatment of rheumatoid arthritis, cardiovascular disease, and many other autoimmune disorders which is becoming a very frequently growing class of novel therapeutics [10]. Biopharmaceutical product which is expressed from the plant through biopharmaceutical production pipeline can be assayed by using proteomics and mass spectrometry. Correct folding and PTMs are the methods that are deployed for stability, maintaining from harvesting up to final delivery. Although, the protein fold correction and quantification in the field of proteomic analysis are the most controversial steps.

2. MATERIALS AND METHODS:

2.1. Application of proteomics in the pharmaceutical industry:

For the last 30 years, biopharmaceutical products have undergone severe advancement and development to find a better cure for some serious diseases. In comparison with conventional and traditionally synthesized pharmaceuticals, potential drugs discovered with the help of proteome analysis can cure disease to a better extent showing lesser side effects [11]. In 1982 insulin became the first approved drug which initiated the approval of so many other biological drugs since then. According to the industrial perspective, over 90 billion dollars have been earned throughout the biopharmaceuticals in revenue as per the record of 2010. The revenue earned is expected to increase almost double by the end of 2022 although the rate of FDA-approved drugs is becoming lesser in the amount [12].

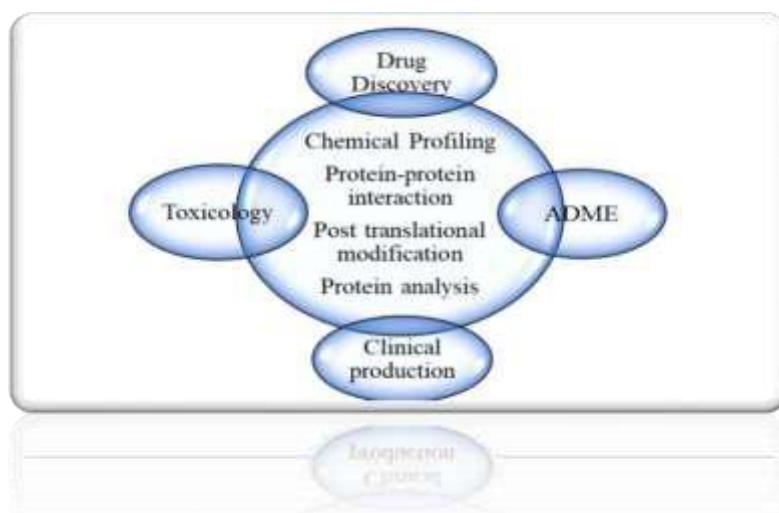


Figure 1: Various application of proteomics in the pharmaceutical industry

The constant rate of increasing application of proteomics in the pharmaceutical industry put a significant impact on the global market. Almost twenty years from today two-dimensional electrophoresis (2D-PAGE) used to be the gold standard for identifying various expressed proteins among control samples and the disease in drug discovery research but there are some research limitations due to which proteomics have not been deployed in the field of drug discovery yet. In mass spectrometry, there are such improvements occurred such as improved resolution, accuracy, and sensitivity which have triggered the proteomics approaches in the drug discovery field. So now, proteomics has become a huge part of the pharmaceutical industry upon integrating with biopharmaceuticals. In a phenotypic screening of drug discovery process, chemical profiling has become a significant part which is also been used to

precise the molecular mechanism of the targeted drug in the screening of phenotypes [13][14]. To identify and enumerate disease and drug-related proteins targeted protein quantification models have been deployed. There are several applications of proteomics that are being used among various sections of pharmaceutical companies for example drug ADME (absorption, distribution, metabolism, and excretion); toxicity, and manufacturing control as depicted in (Fig. 1). Recent proteomics reports suggested that several trends in proteomics application have significantly modified the pharmaceutical market along with research and development.

2.2. Role of proteomics in drug discovery:

Proteomics study along with mass spectrometry analysis has elongated its role in the diverged way throughout the research field. Drug discovery is a complex process with high cost and newly emerging technologies, proteomics can help to spread and accelerate the methods of the discovery process [15]. The study says that each new drug candidate costs more than 70 million dollars. Drug discovery is a multidisciplinary phase that uses genomics, metabolomics alongside proteomics involving several stages as depicted in the Fig. 2. From the Fig.2. There are several stages in the drug discovery process among which proteomics plays a significant role where drug action, toxicity, and its efficacy have gone under proper examination. As gene expression assay is not a determination of protein expression and genes express only the cellular events up to the transcriptome stage, proteomics-based drug discovery is more advanced than genomic- based drug discovery [16][17].



Figure 2: Different stages of drug discovery based on proteomics

Proteomics study can measure proteins concerning the post-translational modifications in a greater amount. In a drug discovery pipeline, the early detection and validation of a disease-associated target is the initiation which is also known as target identification. It is clear that proteomics plays a significant role in identifying target proteins which are the main drug targets [18]. The small molecules or the ligands act on the protein as their respective targets with protein-protein interactions creating a cellular network. To understand the cellular network of protein interactions, the proteomics-based approach is a significant step towards the discovery of drug candidates on their protein targets. Clinical, functional, and chemical proteomics is, being part of several mass spectrometry-based platforms, involved in the drug discovery process. The study of the interaction between drug and target molecule can be enabled via these several methods in the modern drug discovery pipeline work-frame. Thus, a new drug molecule can be elucidated by obtaining the selectivity and specificity, drug activity including efficacy, resistance and toxicity, and mechanism of action of the drug [19]. One of the important branches among several methods is functional proteomics which deals with the explanation of the cellular function and mechanism of the unknown protein at the molecular level which helps to light up the way for evolving new drugs. This method is applicable to identify function, activity, and interaction of the abundant proteins including PTMs which enable the understanding of biological systems [20]. This approach is applied to investigate many drug targets for pathological conditions and as well as to achieve information on signalling pathways of proteins and interaction of drug with proteins. The data obtained through this method will be helpful to characterize the special group of proteins in response to signals.

2.3. Use of biomarker for diagnostic purpose:

Clinical diagnosis and regular monitoring activity of the disease progression and development can be indicated by a biochemical indicator refers to a disease-related protein which is known as a biomarker, guiding through the molecular target treatment or development of the clinical response [21]. In the field of medical science,

the biomarker is used as a traceable substance that is used to detect health conditions of any organ function for example prostate-specific antigen is used as a frequently used biomarker in medicine. Biomarkers are very useful in cancer prognosis where malignancies are usually detected and very less treatment options are left for the patients which are often due to high cost and time-consuming process [22]. However, to detect early malignancies a better analysing system is needed to be developed with the amalgamation of several onco-proteome data. The Fig.3. describes the several steps which are to be deployed when working with a biomarker. In the case of a disease, the expression of the proteins is altered, and detecting and monitor those altered proteins in biological samples can assist to give the indicators of the disease [23]. Biomarker discovery is assisted by the development of molecular medicines by using this proteomics technology to a large extent which can easily identify disease-specific biomarkers. Biomarker when used as a disease diagnosis, it is associated with the detection of the disease. On the other hand, prognostic biomarker helps to collect the data of patient response upon giving a drug and thus recurrence of the disease can be predicted. To classify the patients into responder and non- responder groups, predictive biomarkers are used. The discovery of biomarkers in the view of proteomic analysis involves cancer disease progression. To diagnose the disease and observe the treatment, it is evident to achieve specific biomarkers of a tumour cell as the proteome signifies the original state of the organ or the cell [24].

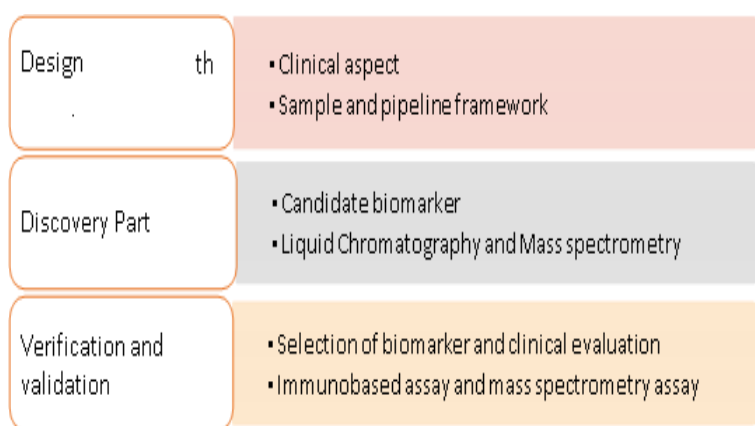


Figure 3: Different steps of new biomarker development. The majority of methods for the analysis of disease-specific biomarkers are based on mass spectrometry (MS).

Heterogeneity between patients is one of the significant limitations in the way of biomarker discovery. Thus, generic medicine or personal medicine by the pharmaceutical industry became the saviour drug for cancer patients. Sensitivity and specificity can be increased by the combined use of biomarkers along with panels to evaluate the response of the patients towards the clinical manifestation [25].

2.4. Proteomics and biopharmaceuticals:

Pharmaceutical industries are extending and developing based on biopharmaceutical products that have higher bioavailability and efficacy than other conventional drugs. Almost all protein purification methods are adopted such as affinity, gel filtration, ion exchange, and reverse-phase chromatography to get a purified form of the biopharmaceuticals from the host plant [26]. In the purification pipeline, some approaches can be complex to compromise the purest form and function of the biopharmaceuticals. In a particular method, the desired protein fraction is highly concentrated in the preparative phase and the method is known as non-chromatographic procedures. Antibodies can be prepared with the help of polymer-salt or polymer-polymer fraction by applying the aqueous-two phase process [27]. The two phases are prepared with PEG-rich phase and salt-rich phase. The concentration of the antibody in the PEG phase can be altered by increasing the salt phase concentration or by decreasing the molecular weight of PEG. To achieve desired biological activity of the biopharmaceuticals many other aspects are important to be considered such as PTMs, protein-protein interaction, protein- ligand interaction. The structure of the biopharmaceuticals is considered to control the efficacy, safety, and immunogenicity of the drug. In the case of sample preparation, X-ray crystallography and nuclear magnetic resonance are considered simpler forms to study protein folding. However, the process is partially correct as data analysis might be exhaustive, and thus false-positive results can be achieved. HDXMS methods are being also used to investigate protein conformation and interaction with their target receptors [28]. The HDX-MS method has a significant impact in studying the alkylation of a protein when it is meant to be degraded. In Fig.4. the steps to obtain a good high yield biopharmaceutical product are depicted. Using the proteomics method of analysis, the specific protein can be quantified and identified to keep the better quality persistent. Protein folding is the major step in the formulation production of biopharmaceuticals.

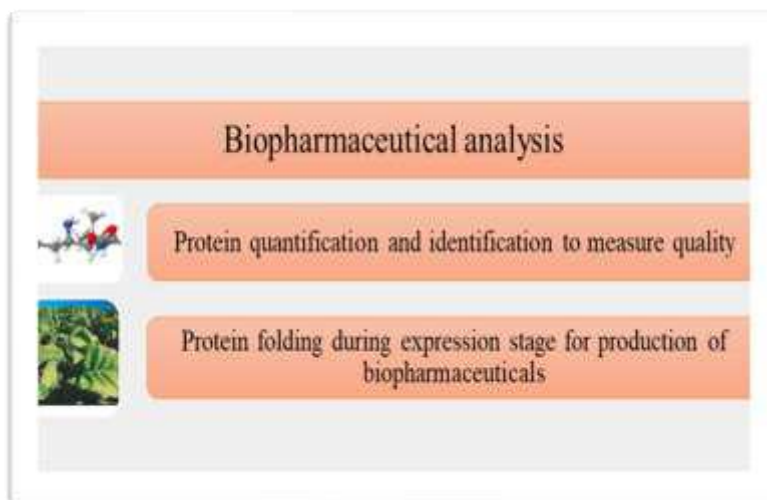


Figure 4: Criteria to analyse the biopharmaceuticals

3. RESULT FROM THE ANALYSIS:

During the year 2014 to 2019, the worldwide proteomics market expanded at a Compound Annual Growth Rate (CAGR) of around 15% which is the study of proteomes with the structure and physicochemical functions on a large scale [29]. The proteomics term was first coined by Mark Wilkins in 1994 where the word proteome suggests the proteins translated from the genomes by synthesizing a whole set of proteins inside a living organism. The existing proteins may have undergone several modifications and alterations while exposing to varying conditions. The study of proteomics deals with several technologies for detection and quantification of protein identifying the diagnostic markers which can help to get a view of protein pathway with pathogens present in the cell. The application of drug discovery and development of generic medicines can be handled through the evaluation of protein production and the interacting patterns of proteins by the knowledge of proteomics. To drive the profitable market the key factor is research and development in the field of drug discovery and personalized medications. These proteomics approaches reduce the time, cost, and resources required for chemical synthesis and biological drug testing to simplify the process of drug discovery. It is needless to say the opportunities related to biomarker identification and mass spectrometry-based proteomics analysis are moving the market growing furthermore [30]. Additionally, the market is driven by increasing technological penetration in the comprehensive analysis of protein techniques.

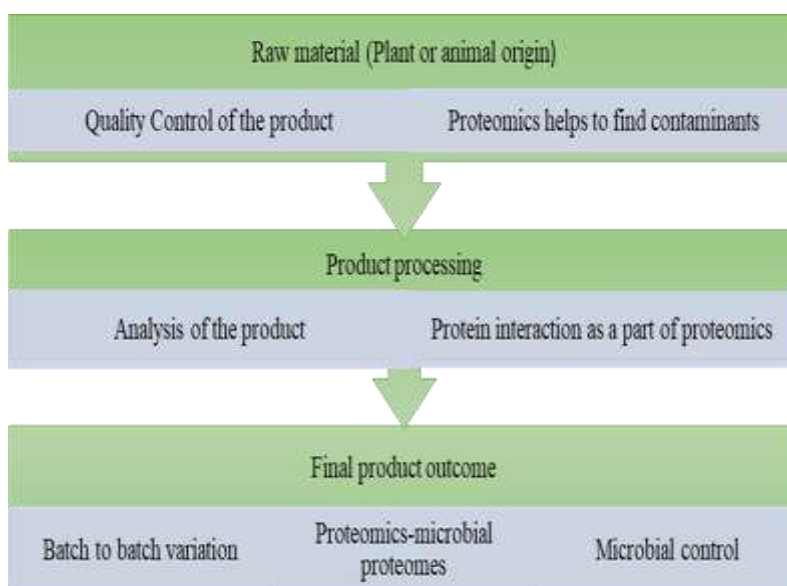


Figure 5: The key steps involved in proteomics analysis of a drug compound

Fig.5. represents the cascade pathway of applying the proteomics approach in the field of drug discovery. The conventional drug discovery model could take 10 to 20 years whereas by this approach the targeted drug discovery can be reached only in 3 to 4 years, by using the proteomics analysis the contaminants and quality control of the developed product could be marked which would further analyse the product by deploying protein interaction. In a large-scale industry, the final modelled product can be achieved by batch variation and microbial proteome. Thus, the

modelled drug can be could be a new benchmark to the pharmaceutical industry which sets a goal to increase the profitability rate of the world economy. The proteomics study deals with the applications of these instruments such as Electrospray Ionization-Liquid Chromatography-Mass Spectrometry (ESI-LC- MS), surface plasma resonance, microarray, protein fractionation systems, and X-ray crystallography in the field of industrial exposure. The advancement of proteomics study is only possible through qualitative and quantitative analysis of proteomes in the identification of cancer therapies. Several other factors like favourable government policies for extensive research and development can also influence the growth of the market. It is to be expected by the IMARC group that the market would continue its growth up to the next five years. The advancement of proteomics study drives the market into the competitive landscape with some major companies like Agilent Technologies Inc., Bio-Rad Laboratories Inc., Creative Proteomics, GE Healthcare Inc., Horiba Ltd., Luminex Corporation, Merck Group, Promega Corporation, Thermo Fisher Scientific Inc., Waters Corporation, etc. By 2027, the global proteomics market is estimated to be worth USD 57.57 billion. The proteomic method cuts down on the time and money required for biological testing and drug synthesis. The rise in the prevalence of genetic disorders, the availability of large amounts of funding for research, and technological advances are all moving the global proteomic market forward. It has a lot of uses in drug discovery and personalized medicine. The proteomic method cuts down on the time and money required for biological testing and drug synthesis. It also streamlines the drug development process. Another factor expected to drive growth is the opportunities associated with mass spectrometry-based proteomics, biomarker recognition, and nano proteomics [31]. Several industry players are trying to increase nanotechnology applications to achieve better performance, precision, and productivity, which will accelerate economic development. Proteomics instruments are inexpensive, which forbids them from being embraced by small businesses and academic institutions. The high- cost factor also makes it more difficult to widen the proteomic market in underdeveloped nations. Chromatography is a powerful tool in a proteomic method that helps for the separation of macromolecules with high resolution, speed, and sensitivity. Besides this, the ability to identify low-abundance specimens is a unique feature of chromatography, which will accelerate demand in the coming years. Due to the diverse application of disease biomarker discovery, targeted therapy, and drug recognition, the clinical diagnosis segment's revenue is rapidly increasing. Proteomics market growth would be aided by technological advancements and the exponential increase of precision medicine. Owing to a growth in the proportion of pharmaceutical firms and research organizations in Asia Pacific, industry revenue is expected to increase at a greater rate. The presence of less strict regulations in the sector also would encourage the demand to flourish.

4. CONCLUSION:

Proteomics holds great potential for drug discovery and development by analysing clinically underlying metabolic activities. Molecular signatures of diseases focused on protein pathways and signalling cascades are currently being identified using new forms of proteomics approaches along with applied bioinformatics. By implementing their discoveries into reality, we will develop an understanding of the functions of individual proteins or cellular pathways. Assessing the cellular circuitry in ongoing molecular networks is predicted to become a beneficial clinical method for personalized medical treatment. With the availability of DNA microarray analysis, that enables for such simultaneous monitoring of thousands of genes, one might wonder why proteomics is so relevant. The proteome's impact cannot be exaggerated, as it is the proteins that provide structure, produce energy, and facilitate interaction, movement, and reproduction within the cell. Proteins, in a nutshell, proteins provide a structural and functional structure for cell life. The protein complement of a cell is dynamic, whilst the genetic information is static. Protein studies raise several issues that are not related to the study of nucleic acids. Dealing with proteins is far more complex than interacting with DNA and RNA. They have secondary and tertiary structures, that would often be preserved during the study. Enzymes, sun, light, and aggressive mixing, such as mixing egg whites, all can denature proteins. Some proteins are difficult to analyse due to their poor solubility. Routine proteomics analysis in the clinic also seems to face a variety of methodological challenges. The implementation of methodologies and the distribution of proteomics data into freely accessible databases are beginning to address these obstacles. Proteomics' expense is also a barrier to its extensive utilization in clinical laboratories. The majority of proteomics technologies rely on sophisticated equipment, high processing speed, and costly consumables. Integration of proteomics, genomics, and metabolomics data, as well as their practical analysis following clinical results, will be another major challenge. Proteomics research provides a depth of information that is entirely complementary to the genetic data generated by genomics research. Proteomics plays an important role in the progress of functional genomics. The combination of genomics and proteomics will play a significant role in biomedical research, as well as in the advancement of diagnostic and therapeutic tools. The integration of genomics and proteomics will have a substantial impact on the yield of diagnostic and therapeutic drugs in the treatment, and it will play a significant role in biomedical research.

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7. CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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