Journal of Bioequivalence & Bioavailability
ISSN: 0975-0851

Atanu Kumar et al., J Bioequiv Availab 2011, 3:11
DOI: 10.4172/jbb.1000099

Review Article Open Access

Integrated Application of Bioprocess Engineering and Biotechniques for Quality & Bulk Drug Manufacturing

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Abstract

One of the major challenges facing the pharmaceutical industry today is finding new ways to increase productivity, decrease costs whilst still ultimately developing new therapies that enhance human health. Both Biotechniques & Bioprocess engineering are integrated for bulk drug manufacturing of biopharmaceutical products to outcome the above problem. Different biotechnological processes are used in industries for large scale production of biological products for optimization of yield and the quality of end product. Various recent developments in biotechniques and its collaborated work with bioprocess engineering helps to maintain the quality in manufacturing process. However in this review article, we systemically describe all the techniques and different bioprocess used for manufacturing of drugs. To the author's knowledge this review represents the most exhaust description of different biotechniques, different bioprocess, process development and design criteria in bioprocess.

Keywords: Bioprocess engineering; Biotechniques; Biopharmaceuticals

Introduction

Recent advances in analytical instrumentation, biosensors and computer technology have lead to the development of new approaches and techniques to control and optimize bioprocesses. Use of on-line measurements to monitor bioprocesses for identifying physiological state of cells and controlling environmental conditions to maintain the cells in the optimal physiological state has become more prevalent. Novel control strategies, along with new concepts, such as expert systems and artificial neural networks have been introduced and applied in bioprocess monitoring and control. The bottleneck, however, is the unavailability of simple, reliable and robust on-line and *in situ* measurement probes and on-line sampling devices, especially for industrial applications. Further development in this field will strongly depend on the improvement of these basic on-line tools [1].

The advancement of bioprocess monitoring will play a crucial role to meet the future requirements of bioprocess technology. Major issues are the acceleration of process development to reduce the time to the market and to ensure optimal exploitation of the cell factory and further to cope with the requirements of the Process Analytical Technology initiative. Due to the enormous complexity of cellular systems and lack of appropriate sensor systems microbial production processes are still poorly understood. This holds generally true for the most microbial production processes, in particular for the recombinant protein production due to strong interaction between recombinant gene expression and host cell metabolism [2]. Therefore, it is necessary to scrutinize the role of the different cellular compartments in the biosynthesis process in order to develop comprehensive process monitoring concepts by involving the most significant process variables and their interconnections.

Although research for the development of novel sensor systems is progressing their applicability in bioprocessing is very limited with respect to on-line and in-situ measurement due to specific requirements of aseptic conditions, high number of analytes, drift, and often rather low physiological relevance. A comprehensive survey of the state of the art of bioprocess monitoring reveals that only a limited number of metabolic variables show a close correlation to the currently explored chemical/physical principles. In order to circumvent this unsatisfying situation mathematical methods are applied to uncover "hidden" information contained in the on-line data and thereby creating correlations to the multitude of highly specific biochemical off-line data. Modelling enables the continuous prediction of discrete off-line data whereby critical process states can be more easily detected. The challenging issue of this concept is to establish significant on-line and off-line data sets. In this context, online sensor systems are reviewed with respect to commercial availability in combination with the suitability of offline analytical measurement methods [1,2]. In a case study, the aptitude of the concept to exploit easily available online data for prediction of complex process variables in a recombinant *E. coli* fed-batch cultivation aiming at the improvement of monitoring capabilities is demonstrated. In addition, the perspectives for model-based process supervision and process control are outlined.

Biotechniques is a sub-discipline of *analytical chemistry* covering the quantitative measurement of xenobiotics (drugs and their metabolites, and biological molecules in unnatural locations or concentrations) and biotics (macromolecules, proteins, DNA, large molecule drugs, metabolites) in biological systems. Many drugs endeavors are dependent upon accurate quantification of drugs and endogenous substances in biological samples; the focus of bioanalysis in the pharmaceutical industry is to provide a quantitative measure of the active drug and/or its metabolites for the purpose of *pharmacokinetics, toxickinetics, bioequivalence* and *exposure–response* [3]. Bioprocess engineering is a con-

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Received December 1, 2011; **Accepted** December 24, 2011; **Published** December 26, 2011

Citation: Atanu Kumar J, Mahalakshmi DK, Sridhari G (2011) Integrated Application of Bioprocess Engineering and Biotechniques for Quality & Bulk Drug Manufacturing. J Bioequiv Availab 3: 277-285. doi:10.4172/jbb.1000099

glomerate of mathematics, biology and industrial design, and consists of various spectrums like designing of fermentors, mode of operations fermentors. It also deals with studying various biotechnological processes used in industries for large scale production of biological product for optimization of yield and the quality of end products. Bioprocesses have become widely used in several fields of commercial biotechnology, such as production of enzymes (used, for example, in food processing and waste management) and antibiotics. As techniques and instrumentation are refined, bioprocesses may have applications in other areas where chemical processes are now used [4]. Because bioprocesses use living material, they offer several advantages over conventional chemical methods of production: they usually require lower temperature, pressure, and pH (the measure of acidity); they can use renewable resources as raw materials; and greater quantities can be produced with less energy consumption. In I most bioprocesses, enzymes are used to catalyze the biochemical reactions of whole microorganisms or their cellular components. The biological catalyst causes the reactions to occur, but is not itself changed. After a series of such reactions (which take place in large vessels called fermentors or fermentation tanks), [1,3] the initial raw materials are chemically changed to form the desired end product. Although it sounds quite simple, this procedure presents two major challenges. Most bioprocesses, enzymes are used to catalyze the biochemical reactions of whole microorganisms or their cellular components. The biological catalyst causes the reactions to occur, but is not changed itself. After a series of such reactions (which take place in large vessels called fermenters or fermentation tanks), the initial raw materials are chemically changed to form the desired end product. Although it sounds quite simple, this procedure presents two major challenges [5].

In this review, we provide a comprehensive discussion of various bioprocess steps, analytical instrumentation, developed to date, and we will discuss these process & techniques separately based on the feature space used and the systematic approaches to quality & bulk drug manufacturing. This includes different analytical techniques at present, concept on bioprocess development, importance to quality manufacturing. To authors' knowledge, this review represents the most exhaustive description of different biotechniques, different bioprocess, process development and design criteria in bioprocess.

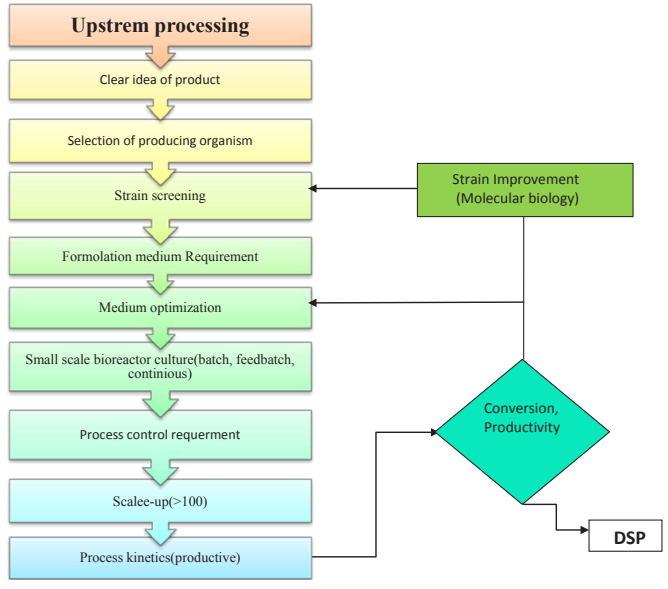

Figure 1: Steps involves in upstream processing for bulk drug manufacturing

Concept of Bioprocess in Bulk Drug Manufacturing

Importance of bioprocess development

The basis of bioprocess engineering involves a careful understanding of the conditions most favoured for optimal production, and the duplication of these conditions during scaled- up production. Advances in genetic engineering have, over the past two decades, generated a wealth of novel molecules that have redefined the role of microbes, and other systems, in solving environmental, pharmaceutical, industrial and agricultural problems. While some products have entered the marketplace, the difficulties of doing so and of complying with Federal mandates of: safety, purity, potency, efficacy and consistency have shifted the focus from the word genetic to the word engineering. The basic concept behind all these is to delivered quality product to market [6].

Design criteria

The design criteria depend on various things like different reactor, concentration, productivity, yield/conversion, quality which leads to purity, sequence, glycosylation and activity [7,8]. Different reactor are used according to the product and organism. The concentration should be in right amount so that there will be no problem in large scale production. [9].

Design criteria for pharmaceutical product

The design criteria for pharmaceutical product are done with high observation. These process focus on four important things(1) Quality (2)concentration (3)productivity (4)Yield/ conversion[10]. By keeping these entire factors in design process the Pharmaceutical product are manufacture.

Design criteria for bulk product

The similar above process is followed for delivering bulk product. This Process is generally done with automation. Both *online* and *off-line* monitoring systems are attached for manufacturing quality product [11].

Unit of bioprocess

The bioprocess engineering has two integral units (1) upstream processing (2) Downstream processing. These two system leads to bulk drug manufacturing.

Upstream processing

The upstream part of a bioprocess refers to the first step in which biomolecules are grown, usually by bacterial or mammalian cell lines, in bioreactors (figure 1). When they reach the desired density (for batch and fed batch cultures) they are harvested and moved to the downstream section of the bioprocess [9,11].

Bioreactor

A bioreactor may refer to any manufactured or engineered device or system that supports a biologically active environment. In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic [12]. These bioreactors are commonly cylindrical, ranging in size from liters to cubic meters, and are often made of stainless steel. The design of bioreactor is accomplish with from small scale to pilot scale.

Mode of operation

On the basis of mode of operation, a bioreactor may be classified as continuous stirred-tank reactor model, batch, fed batch or Bubble column [13].

Continuous stirred tank bioreactor: This requires a relatively high input of energy per unit volume. Baffles are used to reduce vortexing. A wide variety of impeller sizes and shapes is available to produce different flow patterns inside the vessel; in tall fermentors, installation of multiple impellers improves mixing. Typically, only 70-80 % of the volume of stirred reactors is filled with liquid; this allows adequate headspace for disengagement of droplets from exhaust gas and to accommodate any foam which may develop. Foam breaker may be necessary if foaming is a problem. It is preferred than chemical antifoam because the chemicals reduce the rate of oxygen transfer. The aspect ratio (H/D) of stirred vessels varies over a wide range. When aeration is required, the aspect ratio is usually increased [11,12,14]. This provides for longer contact times between the rising bubbles and liquid and produces a greater hydrostatic pressure at the bottom of the vessel.

Batch reactor: The Batch reactor is the generic term for a type of vessel widely used in the process industries. Its name is something of a misnomer since vessels of this type are used for a variety of process operations such as solids dissolution, product mixing, chemical reactions, batch distillation, crystallization, liquid/liquid extraction and polymerization [15]. A typical batch reactor consists of a tank with an agitator and integral heating/cooling system. These vessels may vary in size from less than 1 liter to more than 15,000 liters. They are usually fabricated in steel, stainless steel, glass lined steel, glass or exotic alloy. Liquids and solids are usually charged via connections in the top cover of the reactor. Vapors and gases also discharge through connections in the top. Liquids are usually discharged out of the bottom [15,16].

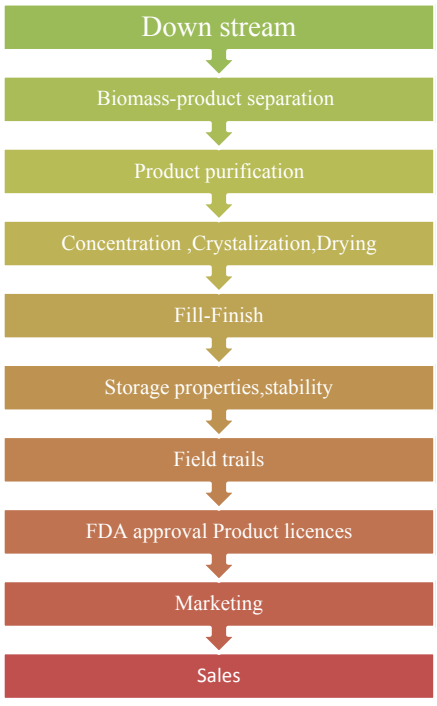

Figure 2: Steps involves in downstrem processing

Fed batch: A fed-batch is a biotechnological batch process which is based on feeding of a growth limiting nutrient substrate to a culture. The fed-batch strategy is typically used in bio-industrial processes to reach a high cell density in the bioreactor. Mostly the feed solution is highly concentrated to avoid dilution of the bioreactor. The controlled addition of the nutrient directly affects the growth rate of the culture and allows to avoid overflow metabolism [17] (formation of side metabolites, such as acetate for *Escherichia coli*, lactic acid in cell cultures, ethanol in *Saccharomyces cerevisiae*), oxygen limitation (anaerobiosis). Substrate limitation offers the possibility to control the reaction rates to avoid technological limitations connected to the cooling of the reactor and oxygen transfer. Substrate limitation also allows the metabolic control, to avoid osmotic effects, catabolite repression and overflow metabolism of side products.

Bubble column: In bubble-column reactors, aeration and mixing are achieved by gas sparging; this requires less energy than mechanical stirring. Bubble columns are applied industrially for production of bakers' yeast, beer and vinegar, and for treatment of wastewater. A height-to-diameter ration of 3:1 is common in bakers' yeast production; for other applications, towers with H/D of 6:1 have been used. The advantages are low capital cost, lack of moving parts, and satisfactory heat and mass transfer performance. Foaming can be problem. Homogeneous flow: all bubbles rise with the same upward velocity and there is no back-mixing of the gas phase. Heterogeneous flow: At higher gas velocity. Bubbles and liquid tend to rise up in the centre of the column while a corresponding down flow of liquid occurs near the walls [18].

Downstream Processing

Downstream processing refers to the recovery and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or fermentation broth, including the recycling of salvageable components and the proper treatment and disposal of waste (Figure 2). It is an essential step in the manufacture of pharmaceuticals such as antibiotics, hormones (e.g. insulin and human growth hormone), antibodies and vaccines; antibodies and enzymes used in diagnostics; [19] industrial enzymes; and natural fragrance and flavour compounds. Downstream processing is usually considered a specialized field in biochemical engineering, itself a specialization within chemical engineering, though many of the key technologies were developed by chemists and biologists for laboratory-scale separation of biological products [20].

Stage of Downstream

Removal of insolubles

This is the first step and involves the capture of the product as a solute in a particulate-free liquid, for example the separation of cells, cell debris or other particulate matter from fermentation broth containing an antibiotic. Typical operations to achieve this are filtration, centrifugation, sedimentation, precipitation flocculation, electro-precipitation, and gravity settling [21]. Additional operations such as grinding, homogenization, or leaching, required to recover products from solid sources such as plant and animal tissues are usually included in this group. *Filtration* is commonly the mechanical or physical operation which is used for the separation of solids from fluids (liquids or gases) by interposing a medium through which only the fluid can pass. *Centrifugation* is a process that involves the use of the centrifugal force for the sedimentation of mixtures with a centrifuge, used in industry and in laboratory settings. More-dense components of the mixture migrate

away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis. Chemists and biologists may increase the effective gravitational force on a test tube so as to more rapidly and completely cause the precipitate to gather on the bottom of the tube [15,22]. *Flocculation*, in the field of chemistry, is a process wherein colloids come out of suspension in the form of floc or flakes by the addition of a clarifying agent. The action differs from precipitation in that, prior to flocculation, colloids are merely suspended in a liquid and not actually dissolved in a solution. In the flocculated system, there is no formation of a cake, since all the flocs are in the suspension.

Product isolation

Product isolation is the removal of those components whose properties vary markedly from that of the desired product. For most products, water is the chief impurity and isolation steps are designed to remove most of it, reducing the volume of material to be handled and concentrating the product. Solvent extraction, adsorption, ultra filtration, and precipitation are some of the unit operations involved [23].

Product purification

This is done to separate those contaminants that resemble the product very closely in physical and chemical properties. Consequently steps in this stage are expensive to carry out and require sensitive and sophisticated equipment. This stage contributes a significant fraction of the entire downstream processing expenditure. Examples of operations include affinity, size exclusion, reversed phase chromatography, crystallization and fractional precipitation [24].

Product polishing

This describes the final processing steps which end with packaging of the product in a form that is stable, easily transportable and convenient. Crystallization, desiccation, lyophilisation and spray drying are typical unit operations. Depending on the product and its intended use, polishing may also include operations to sterilize the product and remove or deactivate trace contaminants which might compromise product safety. Such operations might include the removal of viruses or dehydrogenation.

Concept of Biotechniques

Biotechniques/bioanalytical techniques employed for the quantitative determination of drugs and their metabolites in biological fluids, plays a significant role in the evaluation and interpretation of bioequivalence, pharmacokinetic (PK), and toxicokinetic studies. The quality of these studies, which are often used to support regulatory filings, is directly related to the quality of the underlying bioanalytical data. It is therefore important that guiding principles for the validation of these analytical methods be established and disseminated to the pharmaceutical community. There are various bioanalytical techniques (Figure 3) which are used for qualitative & quantitative analysis of pharmaceutical products [25,26].

Different Bioanalytical techniques

Hyphenated techniques

- LC–MS (liquid chromatography–mass spectrometry)
- GC–MS (gas chromatography–mass spectrometry)
- LC–DAD (liquid chromatography–diode array detection)
- CE–MS (capillary electrophoresis–mass spectrometry)

Chromatographic methods

- HPLC (high performance liquid chromatography)
- GC (gas chromatography)
- UPLC (ultra performance liquid chromatography)
- Supercritical fluid chromatography

Ligand binding assays

- Dual polarization interferometry
- ELISA (Enzyme-linked immunosorbent assay)
- MIA (magnetic immunoassay)
- RIA (radioimmunoassay

Liquid chromatography–mass spectrometry (LC-MS)

Analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. LC-MS is a powerful technique used for many applications which has very high sensitivity and selectivity. Generally its application is oriented towards the general detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture) [27]. The limitations of LC-MS in urine analysis drug screening are that it often fails to distinguish

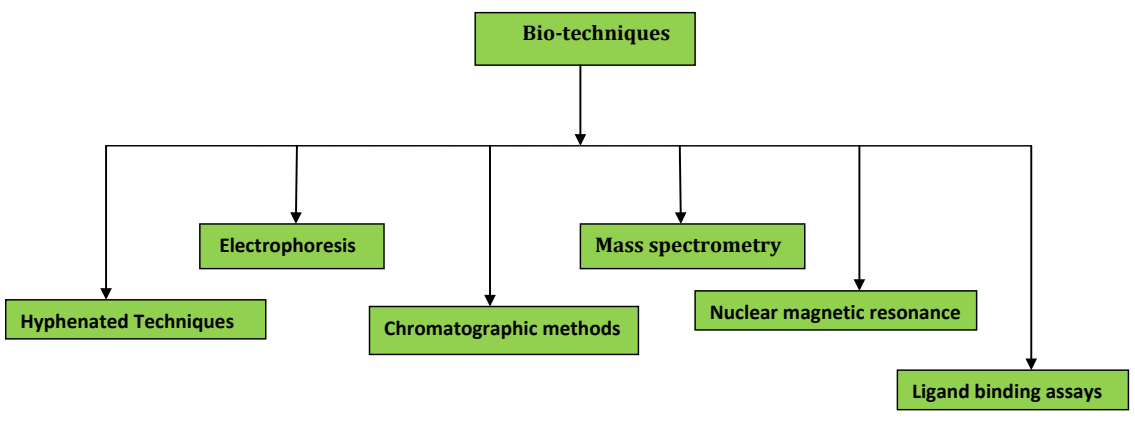

Figure 3: Involvement different biotechniques to maintain quality in downstream processing.

between specific metabolites, in particular with hydrocodone and its metabolites. LC-MS urine analysis testing is used to detect specific categories of drugs however Gas chromatography (GC-MS) should be used when detection of a specific drug and its metabolites is required.

Gas chromatography–mass spectrometry (GC–MS)

Gas chromatography–mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated [28] beyond identification. GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a *specific test*. A specific test positively identifies the actual presence of a particular substance in a given sample. A *non-specific* test merely indicates that a substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the substance, this could lead to false positive identification.

Liquid chromatography–diode array detection (LC–DAD)

Diode array detection is a multi-wavelength detection system that enables the simultaneous collection of absorption data from a range of UV and visible (UV/vis) wavelengths. Scanning and processing of the signals enables 'snapshots' of the complete UV/vis absorption spectrum of the eluent flowing through the LC detector cell to be collected and stored over time. Since many organic compounds have characteristic UV/vis absorption spectra due to the presence of chromophoric groups or structures, this feature can be utilised to assist in identifying the component as it passes through the detector. By simultaneously monitoring a range of wavelengths the absorption spectrum of a compound can be produced and compared with standard spectra from reference compounds [29]. In addition, by selecting the wavelength of maximum absorption improved detection sensitivity can be achieved.

Capillary electrophoresis–mass spectrometry (CE-MS)

This is an analytical chemistry technique formed by the combination of the liquid separation process of capillary electrophoresis with mass spectrometry. Ions are typically formed by electrospray ionization, but they can also be formed by matrix-assisted laser desorption/ionization or other ionization techniques [30]. It has applications in basic research in proteomics and quantitative analysis of biomolecules as well as in clinical medicine.

High performance liquid chromatography (HPLC)

High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column, and a detector to provide a characteristic retention time for the analyte. The detector may also provide additional information related to the analyte, (i.e. UV/Vis spectroscopic data for analyte if so equipped) [31]. Analyte retention time varies depending on the strength of its interactions with the stationary phase, the ratio/composition of solvent(s) used, and the flow rate of the mobile phase. It is a form of liquid chromatography that utilizes smaller column size, smaller media inside the column, and higher mobile phase pressures.

Ultra performance liquid chromatography (UPLC)

UPLC refers to Ultra Performance Liquid Chromatography. It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption. UPLC is comes from HPLC. HPLC has been the evolution of the packing materials used to effect the separation. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5µm, there is a significant gain in efficiency and it's doesn't diminish at increased linear velocities or flow rates [32]. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance.

Supercritical fluid chromatography

The most common separating phases have been liquids and gases. Liquid extraction and liquid chromatography (LC) are methods in which the separating phase is liquid, while in distillation and gas chromatography (GC) the separating phase is a gas. When supercritical fluids are used as the separating phase rather than gases or liquids, the separation processes are called supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) [33].

Application of Bioprocess Engineering in Pharmaceutical

When a product is manufacture in bulk amount, bioprocess engineering plays a important role. Manufacturing product in small scale plant is simple when it come to mass product all the suitable parameter are considered. Production of synthetic amino acid, beverages, vaccines, hormones, antibiotics all these are accomplish with bioprocess engineering.

Streptomyces Venezuelae are aerobic bacteria that are capable of produce jadomycin when shocked by alcohol in a nutrient deprived amino acid rich medium. The size of the bacterial population that is transferred from the growth medium to the production medium can significantly affect the jadomaycin yield. The results showed that the triphenyl formazan was related to the number of cells. Methanol was better able to permeate the cells and extract higher amount of TF than ethanol. The amount of TF increased with the number of extractions for both solvents. A lower medium pH and/or lower temperature produced the highest amount of TF. The best test conditions that produced the highest TF yield were three extractions using methanol after an incubation time of 1 hour at a temperature of 30°C and a medium pH of 6 [34].

The effect of process variable on yield (%w/w protein per biomass) of mycoprotein production by Fusarium venenatum ATCC 20334 in surface culture evaluated. A face centered central composite design (FCCD) was employed to determine maximum protein production at suitable initial concentration of date juice (as a carbon and energy source), nitrogen concentration and seed size. Analysis of variance showed that the contribution of a quadratic model was significant for the response [35]. After reduction of ribonucleic acid contents of mycoprotein, the amino acids and fatty acids profiles of product were determined.

The study investigated the parameter optimization for anthocyanin extraction from egg plant (Solanum melongena) with the approach response surface methodology. Extractions were carried out using acid–

ethanol with the temperature range (60–90°C), time (30–90 min) and solid–liquid ratio (1:15–1:30). Three level three factor Box-Behnken design was followed to observe the anthocyanin yield for the studied parameters. The maximum yield of anthocyanin was observed at the temperature 76.5°C in 70 min in the ratio of 1:26 [36].

A novel attempt is made to convert the calcium carbonate skeleton of widely available garden snail shell (Helix aspersa) to hydroxyapatite based bioceramics [37]. The snail shell was found to decompose within 850°C to all the carbonate phases. The calcined snail shells were then treated with acids followed by different chemicals in ammoniacal media maintaining proper stoichemetry to produce fine Hydroxyapatite (HAP) as filter cake with Ca/P molar ratio of 1.67. The dried HAP powder was extremely pure with specific surface area of 15m2/g. The different characterization techniques were adopted both for calcined snail shell and HAP synthesized like X-ray Diffraction (XRD), Thermal Analysis (DTA/ TGA), Fourier Transform Infra red Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) [38]. The surface area and the particle size, of the HAP powder prepared by chemical precipitation route, were also determined by BET and Malvern particle size analyzer respectively. The synthesized powder was soaked in stimulated body fluid (SBF) medium for various periods of time in order to evaluate its bioactivity. The changes of the pH of SBF medium were measured. High bioactivity of prepared HAP powder due to the formation of apatite on its surface was observed.

Non-molecular systems biology is aimed at the prediction of the functional features of bio-systems on the basis of known cell proteomes and interactomes. Understanding the interactions between all the involved molecules is therefore the key for gaining a deep understanding of such processes. Albeit many thousands of interactions are known, accurate molecular insights are available for only a small fraction of them. The difficulties found in the resolution of atomic level structures for interacting pairs, make the predictive power of molecular computational biology methods essential for the advancement of the field [39]. Indeed, bridging the gap formed due to the lack of structural details can therefore transform systems biology into models that more accurately reflect biological reality.

Xylitol is a fermentation product of xylose that can be used as a sweetener by diabetics. Five novel Kluyveromyces marxianus yeast strains, IMB1, IMB2, IMB3, IMB4 and IMB5, have shown the ability to grow on xylose and produce xylitol. In comparison to chemical synthetic xylitol production, biological production can be more cost effective since no expensive metal catalysts and high temperatures are needed. The five K. marxianus IMB strains were tested at temperatures ranging from 25°C to 45°C with xylose as the sole carbon source. IMB2 was found to be the best xylitol producer among the IMB yeast. Product yields [40,41] (YP/S) up to 0.90 g/g at 40°C with 1.0 g/l initial cell mass and 50 g/l initial xylose concentration were observed for IMB2.

There is an increasing demand worldwide for the application of intelligent, fast and inexpensive measurement systems in clinical diagnosis. In the field of Clinical Microbiology, current techniques generally require 24-48 hours to identify and characterize a pathogenic microorganism following a series of biochemical tests. Although new molecular biological and serological test have been introduced recently, they still have not replaced cultural methods and microscopy. Increased capital costs, need of highly skilled personnel and contamination, reduces the efficiency of these methods in the diagnosis of diseases like H.pylori infection and Tuberculosis [42].

The production media used to produce Penicillin G was sterilized at various time intervals and the change in penicillin production along with the level of precursors utilized by the P. chrysogenum were analysed. The change in sterilization time varied the proportion of fermentation media converted from complex to simple. Studies were carried out in both shake flask level and also in laboratory scale fermenter (3 litres) with media containing PAA (Phenyl acetic acid) as precursor. The fermentation media used in this study contained K_2SO_4, KH_2PO_4, $(NH_4)_2SO_4$, corn steep liquor (N_2 source), Lactose (Carbon source) and $CaCO_3$ [43]. The steam batch sterilisation at 121°C was attempted with different time intervals between 25 to 50 minutes with 5 minutes increment. It was observed that change in the sterilization time increased the Penicillin-G production by 30 % upto 30 minute and only 6% upto 45 minute and then it started to drop. HPLC method was used to carry out quantitative analysis of the product Penicillin G and the precursor Phenyl acetic acid [44,45]. The results further concluded that though the rise in the sterilization temperature increased the Penicillin G production rate, it was cost effective as more energy required to rise the sterilization temperature which in turn increased the cost of production of Penicillin G.

Application of Biotechniques in Pharmaceutical

Morphine and oxycodone are widely used as analgesic drugs for cancer pain. The pharmacological effects of these drugs and also their metabolites have been reported in experimental papers, but in humans, the relationships between these plasma concentrations and the clinical effects remain unclear. Also the necessity for simultaneous determination of both drugs has been suggested because opioid rotation is performed clinically. However, to date there is no study which has simultaneously determined these four drugs, and also achieved a high recovery. In order to perform a reliable pharmacokinetic study of cancer pain patients receiving morphine and oxycodone, an easy, rapid, sensitive and selective analytical method was proposed and validated [46,47].

A rapid and simple method for the separation and quantification of paromomycin sulfate and its impurities by HPLC coupled with evaporative light scattering detection (ELSD) was developed. The chromatographic conditions included the use of a GRACE Alltima C_{18} column (250mm×4.6mm, 5μm) maintained at 30°C and a mobile phase of 0.2 M trifluoroacetic acid water–acetonitrile (96:4, v/v) at a flow rate of 0.6 mL/min[48,49]. The influence of gas pressure and temperature of the drift tube in the detector on the detection response was also investigated.

A rapid and sensitive liquid chromatography/mass spectrometry (LC/MS) method was developed and validated for the determination of saikosaponin a in rat plasma. Saikosaponin a was extracted by protein precipitation with acetonitrile and the chromatographic separation was performed on a C_{18} column. The total analytical run time was relatively short (5.5 min) and the limit of assay quantification (LLOQ) was 10 ng mL^{-1} using 100 μL of rat plasma. Saikosaponin a and the internal standard (felodipine) were monitored in selected ion monitoring (SIM) mode at *m/z* 779.2 and 382.0, respectively [50-52].

Development and subsequent validation of a stability indicating reverse - phase highperformance liquid chromatography method for the simultaneous estimation of salbutamol sulphate and theophylline in tablet dosage forms. A reversed-phase phenomenax C-18 column (250 mm × 8 mm i.d., particle size 10 μm) column with mobile phase consisting of acetonitrile and phosphate buffer 65:35 (v/v) (pH 4.2 ± 0.02, adjusted with triethylamine) was used. [53,54]. The flow rate was

1.2 mL min-1 and effluents were monitored at 235 nm. The retention times (tR) of salbutamol sulphate and theophylline were found to be 5.33 min and 13.36 min, respectively.

Simple, accurate, and reproducible UV spectrophotometric and HPLC method for simultaneous estimation of salbutamol (SAL) and prednisolone (PRE) was developed in the present work. The first developed method was Simultaneous equation method, wavelength selected are 227 nm for salbutamol and 244 nm for prednisolone respectively. Linearity was observed in concentration range of 6-20µg/ml for salbutamol as well as for prednisolone. Second developed method was RP-HPLC method using Thermo C_{18} column (4.6 mm i.d × 250 mm) and acetonitrile: 0.025M potassium dihydrogen orthophosphate buffer (pH adjusted to 3.5 with orthophosphoric acid) in the ratio of 30:70% v/v as mobile phase [55-57]. For HPLC method, linearity was observed in the concentration range of 20-100µg/ml for salbutamol as well as for prednisolone and drugs was subjected to oxidation, hydrolysis, and heat to apply stress condition for degradation studies.

A new simple, precise, accurate and selective TLC-densitometry method has been developed for simultaneous determination of tamsulosin hydrochloride and finasteride in tablet dosage form. Chromatographic separation was performed on aluminum plate precoated with silica gel 60 F_{254} using toluene: n-propanol: triethylamine (3.0:1.5:0.2 v/v) as mobile phase. Detection was carried out densitometrically at 260 nm. The *R*F value of tamsulosin hydrochloride and finasteride were 0.32 and 0.54, respectively. The reliability of the method was assessed by evaluation of linearity which was found to be 200 – 1200 ng/spot for tamsulosin hydrochloride and 1000 - 6000 ng/spot for finasteride. Accuracy of the method was accessed by percentage recovery and found to be 99.77 ± 0.71 % for tamsulosin hydrochloride and 99.75 ± 0.86 % [58,59] for finasteride.

A quick and sensitive reversed phase high performance liquid chromatography (HPLC) method has been developed in Indian surgical patients to determine the concentration of Propofol In human plasma. Propofol can be isolated from human plasma by adding 1ml precipitating solution which consists of acetonitrile and perchloric acid (67:33) mixture, which also contains dibutylpthalate (1mg/ml) as an internal standard. The sample is mixed for two minute on a vortexer. The plasma substance precipitated by acetonitrile and perchloric acid are further separated by centrifugation. The supernatant is directly injected into the HPLC system with the help of autosampler. The analysis was carried out using column 250 × 4.6 mm column packed with10-µm Spherisorb reversed phase octadecyl silane particles (C_{18}) [60,61]. The 500ml of mobile phase (67:33:0.04) consisted of 335ml of acetonitrile and 165ml of distilled water and 200µl of acetic acid maintaining the pH 4.0. The flow rate of the mobile phase was 1.5ml/ min. propofol was monitored by a UV detector at a 270nm wavelength [62,63].

The accumulation of brominated flame retardants (BFRs) in the environment raises concern in light of observed detrimental effects on wildlife as well as on public health. We here present a recently modified method for the identification and quantification of the following selection of bromodiphenyl ether (BDE) flame retardants: BDE-17, -47, -66, -100, -153 and -183, in soil and sediments, using a new extraction procedure followed by gas chromatography mass spectrometry (GC-MS). Low- and high- resolution mass spectrometry (LRMS and HRMS, respectively) were compared and the latter was found to be superior with respect to both sensitivity and linear range. At LRMS mode the linear range was 3.8 – 19.2 ng/g dry weights (dw), while the use of HRMS more than doubled the linear range to 1.9 – 38.4 ng/g dry weight. Both methods were tested with regards to matrix associated effects on the limit of detection and quantitation [64,65]. The use of HRMS yielded equal sensitivity for standards in solution and matrix. This was not the case when using LRMS. Here the limits of detection and quantitation were severely elevated by the matrix. Recoveries were comparable, but slightly higher at LRMS mode (77.0 – 121.9%) compared to HRMS (83.2 – 115.3%). The method described here is high throughput, low cost and will prove valuable in monitoring the levels of BFRs in the environment [66].

A method of analysis of phloretin [3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one] in biological fluids is necessary to study the kinetics of in vitro and in vivo metabolism and its concentration in natural products. A highperformance liquid chromatographic (HPLC) method was developed for the determination of phloretin in rat serum. Separation was achieved on a Chiralcel® OD-RH column with UV detection at 288 nm. The calibration curves were linear ranging from 0.5 to100 µg/ml. The mean extraction efficiency was >95%. Precision of the assay was <14%, and was within 10.9% at the limit of quantitation (0.5 µg/ml). Bias of the assay was lower than 14%, and was within 9.22% at the limit of quantitation [67-69]. The HPLC method was successfully applied to the pharmacokinetic study of phloretin in rats.

Quality control of drug fleroxacin dosage, its monitoring in biological fluids, and research of drug's metabolism and action are an important analytical task. A new chemiluminescence (CL) reaction system was established for the determination of fleroxacin (FLX). The trivalence dysprosium-sensitized CL emission mechanism was investigated by comparing the fluorescence emission with CL spectra. The CL spectra of FLX-$KMnO_4$-$Na_2S_2O_3$-$H_6P_4O_{13}$ system are from the narrow characteristic emission of Dy^{3+} at 482 and 578 nm ($^4F_9 \rightarrow {}^6H_{15/2}$, $^4F_9 \rightarrow {}^6H_{13/2}$) through the energy transfer from the excited SO_2^* to analyte, followed by intramolecular energy transfer from analyte* to Dy^{3+}. The optimum conditions for CL emission were investigated and optimized [70,71]. The relationships between the relative CL intensity and the concentration of the studied analyte have good linearity. The detection limit for FLX was 3.0×10^{-10}g/mL. The relative standard deviation is 2.0% for 11 determinations of FLX at 2.0×10^{-6} g/mL. The proposed CL system has been successfully applied for the determination of FLX in the injections and urine sample with satisfactory result.

Conclusion

The integrals application of bioprocess and biotechniques for bulk drug manufacturing and maintaining the quality of pharmaceutical is highly accessible. While manufacturing a pharmaceutical product it should be cost effective, quality should be maintained at optimum level. In this review article the author tried all the details about the bioprocess & biotechniques step through which manufacturing of pharmaceutical product take place. In the years to come, increasing focus will be given to further exploitation of chemical/physical principles to enhance the spectrum of online/inline techniques and the application of miniaturized sensor systems, e.g. micro machines for process monitoring. This development is driven by the ever increasing needs to improve the efficiency of process development, to implement rational design and pursue the FDA's initiative regarding process analytical technology. Following the goal of PAT the manufacturing process must be better understood and easier to control. Quality cannot be solely derived from the products; it should be built-in or should be by design. Therefore Process Analytical Technology relies strongly on monitoring of physiological

relevant variables, which have to be gained "on-line" by modelling. By application of the modelling approach the discrete offline samples are transformed into continuously available signals, whereby permanent supervision is provided and moreover deviations from predefined states can be identified at early stages. Within the PAT framework, multivariate data acquisition and analysis is an important tool, which highlights the upcoming importance of these methods regarding regulatory affairs. Gains in quality, safety and/or efficiency will vary depending on the product and are, among other points, likely to come from increasing automation to improve operator safety and reduce human errors. To achieve further advances in bioprocess optimization, key process variables describing the potentials and limits of the biological system need to be available online. This approach has the potential to enhance the process monitoring capabilities and to fulfil the upcoming requirements for bioprocess development and operation.

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