THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI-600 032

SYLLABUS – M. PHARMACY 2006-2007
BRANCH VI – PHARMACEUTICAL BIOTECHNOLOGY

M. PHARMACY

I YEAR

SYLLABUS FOR PHARMACEUTICAL BIOTECHNOLOGY – BRANCH VI

COMMON TO ALL BRANCHES - PAPER – I

MODERN PHARMACEUTICAL ANALYTICAL TECHNIQUES

THEORY **75 Hours(3 hrs./week)**

1. UV-VISIBLE SPECTROSCOPY : 6 Hours.

Brief review of electromagnetic spectrum and absorption of radiations. The chromophore concept, absorption law and limitations. Theory of electronic spectroscopy, absorption by organic molecules, choice of solvent and solvent effects, modern instrumentation – design and working principle. Applications of UV-Visible spectroscopy (qualitative and quantitative analysis), Woodward – Fischer rules for calculating absorption maximum, Photometric titrations and its applications.

2. FLAME EMISSION SPECTROSCOPY AND ATOMIC ABSORPTION SPECTROSCOPY: 3 Hours.

Principle, instrumentation, interferences and applications in Pharmacy.

3. SPECTROFLUORIMETRY : 3 Hours.

Theory, instrumentation, advantages, relationship of chemical structure to fluorescence spectra, solvent effect, effect of acids and bases on fluorescence spectra, concentration effects, factors affecting fluorescence intensity, comparison of fluorescence and UV-Visible absorption methods and applications in Pharmacy.

4. INFRARED SPECTROPHOTOMETRY : 6 Hours.

Introduction, basic principles, vibrational frequency and factors influencing vibrational frequency, instrumentation and sampling techniques, interpretation of spectra, applications in Pharmacy. FT-IR-theory and applications, Attenuated Total Reflectance (ATR).

5. NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY : 8 Hours.

Fundamental Principles and Theory, Instrumentation, solvents, chemical shift, and factors affecting chemical shift, spin-spin coupling, coupling constant, and factors influencing the value of coupling constant, spin-spin decoupling, proton exchange reactions, FT-NMR, 2D -NMR, NMDR, NOE, NOESY, COSY and applications in Pharmacy, interpretation of spectra, C13 NMR-Introduction, Natural abundance, C13 NMR Spectra and its structural applications.

6. ELECTRON SPIN RESONANCE SPECTROSCOPY : 2 Hours.

Theory and Principle, Limitations of ESR, choice of solvent, g-values, hyperfine splitting, instrumentation, difference between ESR & NMR and applications.

7. MASS SPECTROSCOPY : 8 Hours.

Basic principles and instrumentation, ion formation and types, fragmentation processes and fragmentation pattern, Chemical ionization mass spectroscopy (CIMS), Field Ionization Mass Spectrometry (FIMS), Fast Atom Bombardment MS (FAB MS), Matrix Assisted laser desorption / ionization MS (MALDI-MS), GC-MS, interpretation of spectra and applications in Pharmacy.

8. X-RAY DIFFRACTION METHODS : 4 Hours.

Introduction, generation of X-rays, X-ray diffraction, Bragg's law, X-ray powder diffraction, interpretation of diffraction patterns and applications.

9. OPTICAL ROTARY DISPERSION : 4 Hours.

Principle, Plain curves, curves with cotton effect, octant rule and its applications with example, circular dichroism and its relation to ORD.

10. THERMAL METHODS OF ANALYSIS : 5 Hours.

Theory, instrumentation and applications of Thermo Gravimetric Analysis (TGA), Differential Thermal Analysis (DTA), Differential Scanning Calorimetry (DSC) and Thermo Mechanical Analysis (TMA).

11. CHROMATOGRAPHIC TECHNIQUES : 15 Hours.

a) Classification of chromatographic methods based on mechanism of separation: paper chromatography, thin layer chromatography, ion exchange chromatography, column chromatography and affinity chromatography – techniques and applications.

b) Gas Chromatography : Theory and principle, column operation, instrumentation, derivatisation methods and applications in Pharmacy.

c) High Performance Liquid Chromatography : Principle, instrumentation, solvents used, elution techniques, RP-HPLC, LC-MS and applications in Pharmacy.

d) HPTLC and Super Critical Fluid Chromatography (SFC) : Theory and Principle, instrumentation, elution techniques and pharmaceutical applications.

12. ELECTROPHORESIS : 3 Hours.

Theory and principles, classifications, instrumentation, moving boundary electrophoresis, Zone Electrophoresis (ZE), Isoelectric focusing (IEF) and applications.

13. RADIO IMMUNO ASSAY : 3 Hours.

Introduction, Principle, Theory and Methods in Radio Immuno Assay, Related Immuno Assay procedures and Applications of RIA Techniques.

14. STATISTICAL ANALYSIS : 5 Hours.

Introduction, significance of statistical methods, normal distribution, probability, degree of freedom, standard deviation, correlation, variance, accuracy, precision, classification of errors, reliability of results, confidence interval, Test for statistical significance – students T-test, F-test, Chi-square test, correlation and regression.

PRACTICALS

1. Use of colorimeter for analysis of Pharmacopoeial compounds and their formulations.
2. Use of Spectro photometer for analysis for Pharmacopoeial compounds and their formulations.
3. Simultaneous estimation of combination formulations (minimum of 4 experiments).
4. Effect of pH and solvent on UV Spectrum of certain drugs.
5. Use of fluorimeter for analysis of Pharmacopoieal compounds.
6. Experiments on Electrophoresis.
7. Experiments of Chromatography.
    (a) Thin Layer Chromatography.
    (b) Paper Chromatography.
        1) Ascending Technique.
        2) Descending Technique.

3) Circular Technique.
4) Two dimensional Paper Chromatography and TLC.

8. Experiments based on HPLC & GC.
9. IR, NMR and Mass Spectroscopy – Interpretation of spectra & Structural elucidation (atleast for 4 compounds each).
10. Any other relevant exercises based on theory.

REFERENCES

1. Spectrometric identification of Organic Compounds, Robert. M. Silverstein et al, 7th Edition, 1981.
2. Fundamentals of Mathematical Statistics, S.C. Gupta and V.K. Kapoor.
3. Principles of Instrumental Analysis by Donglas A. Skoog, James, J. Leary, 4th Edition.
4. Pharmaceutical Analysis – Modern Methods – Part A, Part B, James W. Munson – 2001.
5. Vogel's Text Book of Quantitative Chemical Analysis, 6th Edition, 2004.
6. Chromatographic Analysis of Pharmaceuticals, John A. Adamovics, 2nd Edition.
7. Practical Pharmaceutical Chemistry, Part two, A. H. Beckett & J. B. Stenlake – 4th Edition.
8. Instrumental Methods of Chemical Analysis – B. K. Sharma - 9th Edition.
9. Instrumental Methods of Analysis – Hobert H. Willard, 7th Edition.
10. Organic Spectroscopy – William Kemp, 3rd Edition.
11. Techniques and Practice of Chromatography – Raymond P. W. Scott, Vol. 70.
12. Identification of Drugs and Pharmaceutical Formulations by Thin Layer Chromatography – P. D. Sethi, Dilip Charegaonkar, 2nd Edition.
13. HPTLC – Quantitative Analysis of Pharmaceutical Formulations – P. D. Sethi.
14. Liquid Chromatography – Mass Spectrometry, W. M. A. Niessen, J. Van Der Greef, Vol. 58.
15. Stereo Chemistry – Conformation and Mechanism by P. S. Kalsi, 2nd Edition.
16. Spectroscopy of Organic Compounds by P. S. Kalsi.
17. Organic Chemistry by I. L. Finar Vol. II – 5th Edition.

SYLLABUS FOR PHARMACEUTICAL BIOTECHNOLOGY

BRANCH – VI

PHARMACEUTICAL-BIOTECHNOLOGY

PAPER – II

PHARMACEUTICAL ASPECTS OF MICROBIAL AND CELLULAR BIOLOGY

THEORY **75 Hours(3 Hrs./week)**

1. Bacteria, Fungi and Viruses: Structure, Chemistry and Morphology, Cultural, Physiological and Reproductive features, Methods of isolation, Cultivation and Maintenance, Nomenclature, General classification, Molecular and Genotypic taxonomy. Industrially important micro organisms including Actinomycetes with examples and uses. **16 Hours.**

2. **8 Hours.**

   (a) Basic aspects of cell regulation.

   (b) Bio-energetics and Metabolism – biochemical mechanisms of generating ATP; Fuelling reactions of aerobic and anaerobic organisms.

   (c) Secondary metabolism and its applications.

3. Nucleic acids, the genetic code and protein synthesis: 10 Hours.

Synthesis of DNA – polymerization of nucleotides into DNA – Basic chemical structure, replication and its role in protein synthesis. Synthesis of proteins – the roles of RNA in Translation (mRNA, tRNA and rRNA).

4. Manipulating cells in culture 12 Hours.

(a) Growth of micro organisms in culture pertaining to Bacteria; Principles of microbial nutrition; physical and chemical environment for microbial growth; Batch, continuous and synchronous cultures; Stability and degeneration of microbial cultures.

(b) Growth of animal cells in culture; General procedures for cell culture; nutrient composition; primary, established and transformed cell cultures; applications of cell culture in Pharmaceutical industry and research.

(c) Growth of viruses in culture; Propagation and enumeration; application of above techniques for antiviral screening.

5. Microbial Genetics: 15 Hours.

(a) Genetic organization of prokaryotic and eukaryotic cells; mutagenesis and repair mechanisms; types of mutants; application of mutagenesis in strain improvement; gene mapping of plasmids – types, purification, transfer and applications.

(b) Transformation, Conjugation, Transduction.
(c) Phage genetics – gene organisation, phage mutation and lysogeny.

6. Immunology : 7 Hours.

Cellular basis for immune response, immunity to viruses, bacteria and fungi, immuno-deficiency diseases, hypersensitivity reactions and auto- immune diseases. Immunisation – Active and Passive.

7. Microbial pathology and chemotherapy: 7 Hours.

Identifying features of pathogenic bacteria, viruses and fungi, mechanism of microbial pathogenecity, etiology and pathology of common microbial diseases, currently recommended therapies for common bacterial, fungal and viral infection, mechanism of action of anti- microbial agents and possible sites for chemotherapy.

PRACTICALS

1. Fumigation of aseptic area and air sampling.
2. Morphological study, isolation and characterization of some bacteria and fungi.
3. Methods of preservation of culture.
4. Isolation and primary screening of Streptomyces.
5. Qualitative analysis of potable water.
6. Estimation of microbial load in pharmaceutical excipients and raw materials as per official pharmacopoeia.
7. Construction of UV survival curve and demonstration of dark repair mechanism.
8. Preparation and maintenance of primary cell culture and cell lines.
9. Enumeration of viruses by titration and plaque assay.
10. Determination of cytotoxicity and screening for anti- viral activity of some natural and synthetic products.
11. Induction of mutation, isolation of antibiotic resistant and auxotropic mutants adopting replica plating technique.
12. Isolation of specialized transducing phage.
13. Animal immunization – innoculation, bleeding and antigen- antibody reactions by haemaggulutination – inhibition, neutralization and precipitin reactions.
14. Standardization of inoculum and estimation of MIC by serial dilution and gradient – plate technique.
15. Qualitative and quantitative analysis of anti- microbial agents by ditch – plate method and extinction methods (RWC test).
16. Microbial sensitivity of some human pathogenic isolates against various antibiotics.

REFERENCES

1. General Microbiology: R.Y. Stanier.
2. Essentials and applications of microbiology : Judy Kandal.
3. Microbiology: Pelczar, Reid and Chan.
4. Genetics of Antibiotic producing Microorganisms : G. Sermonti.
5. Microbial Genetics : David Freifelder.
6. Topley & Wilson : Volumes I to IV.
7. Genes V and VI : Lewin Benjamin.
8. Virology : Fields.
9. Animal cell culture : Ian Freshney.
10. Immunology : Weir.
11. Immunology : Ivan Roitt, Johnathan Bronstoff, David Male.
12. Medical Microbiology: Mackie and McCartney.
13. The Actinomycetes : Waksman SA.

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PAPER – III

BIOPROCESS TECHNOLOGY

THEORY **75 Hours(3 hrs./week)**

1. Basic principles in fermentation **8 Hours.**

2. Isolation, Screening and application of industrially important microbes – primary and secondary screening, maintenance of stock cultures, strain improvement for increased yield. **6 Hours**.

3. **6 Hours**.
   a. Detailed study of the design and operation of bioreactor, ancillary parts and functions; impeller design & agitation power requirements; on-line measurement and control of dissolved oxygen, carbon-di-oxide, temperature, pH and foam.
   b. Types of reactors – CSTR, tower, air-lift, bubble-column, packed bed, hollow fibre –configuration and applications.

4. Mass transfer – theory, diffusional resistance to oxygen transfer, oxygen requirements of micro organism, measurement of mass transfer coefficient and factors affecting them; effects of aeration and agitation on mass transfer, supply of air, air compressing, cleaning and sterilization of air, air sampling and testing standards for air purity. **6 Hours.**

5. Rheological properties of fermentation systems and their importance in bioprocessing. **4 Hours.**

6. Fermentation kinetics: **4 Hours.**
   Reaction kinetics : Michaelis Menten constant and Monod equation-derivations for biomass estimation; Lineweaver–Burke plot.

7. Cultivation systems – closed, semi-open and open systems; graphical plots representing the above systems; use of immobilized culture systems to prepare fine chemicals. **4 Hours.**

8. Scale up of fermentation process: **4 Hours.**

   Principles, theoretical considerations and techniques used; fermentation media, HTST sterilization – advantages and disadvantages, liquid sterilization techniques; Thermal death kinetics.

9. Downstream processing: **6 Hours.**

Theory, equipment design and operation, methods, filtration, solvent extraction, chromatographic separation, crystallization, turbidity analysis and cell yield determination metabolic response assay, enzymatic assay, bioautography, techniques for disruption of cells for product recovery.

10. Bioprocessing of the following industrially important microbial metabolites:

15 Hours.

Organic solvent	:	alcohol.
Organic acids	:	Citric acid and lactic acid.
Antibiotics	:	Penicillin, Streptomycin, Griseofulvin Cephalosporins, Amphotericin B, Rifampicin, Mitomycin – C.
Vitamins	:	Vit. B12 and Riboflavin.
Aminoacids	:	Glutamic acid and Lysine.
Nucleotides	:	Cyclic AMP & GMP.

11. Biosynthetic pathways for some secondary metabolites, microbial transformation of steroids and alkaloids. **6 Hours.**

12. Computer control of fermentation processes: **4 Hours.**

System configuration and applications.

13. Regulations governing the manufacturing of biological products. **2 Hours.**

PRACTICALS

1. Isolation and secondary screening of industrially important microorganisms.
2. Strain improvement (for increased yield) by stress inducers.
3. Preparation, calibration, and standardization of a bioreactor.
4. Power calculations, KLa determinations and MTR calculations of a typical bioprocess.
5. Construction of growth curve and determination of specific growth rate and doubling time.
6. Biomass estimation by monitoring protein synthesis and sugar depletion.
7. Enzyme kinetic study
    a. Effect of metal ion concentration.
    b. Effect of pH.
    c. Effect of temperature.
    d. Effect of varying substrate concentration.
    e. Kinetic parameter calculations.
8. Protein separation by aqueous two-phase partitioning.
9. Fermentation process of alcohol and wine production.

10. Fermentation of vitamins and antibiotics.
11. Whole cell immobilization engineering
    a. Using various polymers.
    b. Study of physical characteristics .
    c. Comparison of efficacy of immobilised and free cells.
12. Down stream processing
    a. methods of cell disruption.
    b. typical isolation process for antibiotics.
    c. purification by chromatographic techniques.
13. Microbiological assay of antibiotics.
14. Thermal death kinetics of bacteria and its applications.

REFERENCES

1. Industrial Microbiology : L.E. Casida.
2. Industrial Microbiology : B.M. Miller and W.Litsky.
3. Microbial Technology Vois I & II : H. Peppler.
4. Industrial Biotechnology : Vedpal S Malik and Padma Sridhar.
5. Biochemistry of Industrial Microorganisms, C Rainbow and AH Rose.
6. Biochemical Engineering : F.C. Webb.
7. Biochemical Engineering : R.Steel.
8. Biochemical Engineering Fundamentals : Balley and Ollis.
9. Current protocols in molecular biology, Vols I & II : F.M Asubel, John Wiley Publishers.
10. Biotechnology of antibiotics and other bioactive microbial metabolites : Gianeario Lancini and Rolando Lorenzetti.
11. Biological reaction engineering : I J Dunn, E. Heinzle, J Ingham, J.E. Prenosil.
12. Bioreactor design and product yield : Butterworth and Heinemann.
13. Enzyme assays – a practical approach: Robert Eisenthal and Michael J Danson.
14. Fermentation and biochemical engineering handbook: Henry C Vogel.

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BRANCH-VI

PAPER - IV

ADVANCES IN PHARMACEUTICAL BIOTECHNOLOGY

THEORY **75 Hours(3 hrs./week)**

1. Enzyme Technology

a) Classification, general properties of enzymes, dynamics of enzymatic activity, sources of enzymes, extraction and purification: Applications pharmaceutical, therapeutic and clinical. Production of amyloglucosidase, glucose isomerase, amylase and trypsin. **8 Hours.**

b) Techniques of immobilisation of enzymes and their applications in the industry. Reactors for immobilised systems and perspective of enzyme engineering. **5 Hours.**

2. Animal Biotechnology:

a) Genetic engineering: Techniques of gene manipulation, cloning strategies, procedures, cloning vectors, expression vectors, recombinant selection and screening, expression in E.coli and yeast. **8 Hours.**

b) Site directed mutagenesis, Polymerase chain reaction, and analysis of DNA sequences. Brief study on instrumentation aspect of Gel documentation system and PCR. **6 Hours.**

c) Gene library and cDNA . **4 Hours.**

d) Applications of the above technique in the production of **6 Hours.**
- i) regulatory proteins interferon, interleukins etc.
- ii) Blood products – Erythropoietin.
- iii) Vaccines – Hepatitis – B,
- iv) Hormones – Insulin.

e) Study on controlled and site specified delivery of therapeutic peptides and proteins through various routes of administration. **3 Hours.**

f) Production of useful proteins in transgenic animals and gene therapy. **3 Hours.**

g) Signal transduction, oncogenes and their proteins. **4 Hours.**

h) The human genome product and stem cell research – a brief study.

4 Hours.

3. Immuno Biotechnology:

a) Hybridoma technology – fusion methods for myeloma cells and B-lymphocytes, selection and screening techniques. Production and purification of monoclonal antibodies and their application in clinical diagnosis, immunotherapy and pharmaceutical research. **4 Hours.**

b) Immuno-diagnosis of infectious diseases. **2 Hours.**

c) Vaccinology – Immunopotentiation, adjuvants, living and non-living antigen, newer delivery systems and naked DNA vaccines. New and improved vaccines against Hepatitis-A, Malaria, Typhoid, Experimental HIV-1 vaccines. **6 Hours.**

4. Microbial biotechnology: 6 Hours.

Biotransformation for the synthesis of chiral drugs and sterols. Biodegradation of xenobiotics, chemical and industrial wastes. Production of single-cell protein.

5. Bio-informatics: 3 Hours.

Information theory and biology, redundancy. Networking: Network access, internet and E-mail servers, use of Databases in biology sequence databases for comparisons.

6. Basic statistics: 3 Hours.

Mean, median & mode Standard deviation and standard errors, simple linear regression, basic of significance test, hypothesis test, levels of significance, student‘t’, ‘Chi’ square and goodness of fit.

PRACTICALS

1. Production of extra-cellular enzymes from microbial sources and downstream processing.
    a. Ammonium sulphate precipitation.
    b. Dialysis.
    c. Size exclusion chromatography.
    d. Affinity chromatography.
2. Estimation of some microbial enzymes and quantification in terms of total protein by lowry method.
3. Isolation of Plasmid DNA – Mini – prep and estimation of DNA.
4. Isolation of RNA from microbial sources and its estimation.
5. DNA cloning with different expression vectors and agarose electrophoretic analysis.
6. Transformation techniques with different antibiotic resistance markers.
7. Southern blotting with radioactive and non-radioactive probes.

8. Northern blotting technique.
9. Qualitative analysis of proteins & Estimation of molecular weight of proteins by PAGE techniques.
10. Enzyme-linked immunosorbant assay and western blotting techniques.
11. Hybridoma techniques: Fusion of myeloma and lymphocytes, screening methods and raising Monoclonal antibodies and purification.
12. Development of suitable delivery system for anti-tumour agents, newer vaccines and therapeutic proteins and peptides for site-specific delivery.

REFERENCES

1. Biotechnology – The biological principles: MD Trevan, S Boffey, KH Goulding and P. Stanbury.
2. Immobilisation of cells and enzymes: Hosevear kennady Cabral & Bicker staff.
3. Principles of Gene Manipulating : RW Old and S.B. Primrose.
4. Molecular Cell Biology: Harvey Lodish, David Baltimore, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, James Darnell.
5. Therapeutic Peptides and Proteins; Formulation, processing and delivery systems: Ajay K Banga.
6. Modern Biotechnology : S.B Primrose.
7. Industrial biotechnology : Vedpal S Malik and Padma Sridhar.
8. Immunology : Ivan Roitt, Jonathan Brostoff and David Male.
9. Gene transfer and expression protocols – methods in Molecular Biology, Vol. VII, Edit E.T. Murray.
10. Current protocols in Molecular Biology, Vol.I & II : F.M. Asubel, John Wiley Publishers.
11. Current protocols in cellular biology, Vol.I & II, John Wiley Publishers.
12. Cell Biology, Vol.I, II & III Edited by Julio E Celis.