

**M. Phil. Biochemistry
Syllabus 2013-14**

- I. As per ordinance No. 89 the duration of M. Phil. Course shall be of three semesters.
- II. The M. Phil. Examination shall consist of the following:

I Semester

Course Work I

Paper	Marks
I. Research Methodology and Quantitative Techniques	50
II. Computer Applications	50

Course Work II

Review of published research in relevant field (Written Report)	70
Oral Presentation	30

II Semester

Paper I Research Methodology, Quantitative Techniques and Computer Applications	100
Paper II Analytical Biochemistry	100
Paper III Biochemistry Research III A /III B	100

III Semester

I. Dissertation	100
II. Viva-voce Examination	100
III. Seminar (Two)	100



Paper I: Research Methodology, Quantitative Techniques and Computer Applications

UNIT I

Research-Definition-Importance and Meaning of Research-Characteristic of Research-Types of Research-Steps in Research-Identification, selection and formulation of research problem-Research Design, Conclusion of Research-Formulation of Hypothesis, Research Reports ; Types of Reports-contents-styles of reporting-Steps in drafting reports-Editing the final draft- Evaluating the final draft. Statistics in Research-Hypothesis-Fundamentals of Hypothesis testing-Standard Error-Point and Interval estimates-Important Non-Parametric tests:Sign, Run, Kruskal-Wallis tests and Mann-Whitney test-Parametric tests:Testing of significance-Mean, Proportion, Variance and Correlation-testing for Significance of difference between Means, Proportions, Variances and Correlation coefficient. Chi-square tests-ANOVA-One way and Two way.

UNIT II

Fundamental Laboratory Techniques, Basic principles, working with liquids, Basic laboratory procedures, Principles of solution chemistry, pH and buffer solutions, Instrumental standardization, optimization of procedures.

Introduction, classification of Analytical methods, Types of Instrumental methods, Instruments for analysis. Analog & Digital signals, Planning for laboratory automation. An overview of automatic instruments and instrumentation. Flow Injection Analysis, Discrete Automatic systems.

The investigative approach: Making and recording measurements, SI units and their use, Scientific method and design of experiments for Project work. Analysis and presentation data: Using graphs, presenting data in tables, Descriptive statistics, Statistical tests, Drawing chemical structures, Elementary ideas of chemometrics, Chemo informatics and Computational chemistry

UNIT III

Chemical safety and Disaster Management: Emergency response: chemical spills, radiation spills, biohazard spills, leaking compressed gas cylinders, fires, medical emergency accident reporting, Use of sensors technology for disaster management

General safety: safety equipments, personal protective equipments, compressed gas safety, safety practices for broken glass wares, centrifuge safety, treated biomedical wastes and scientific ethics.

Isolation and Purification of compounds, Solvents for recrystallization, Sublimation, Fractional/vacuum/steam distillation, Limit tests, Sources of errors in observations

Basic principle, operating method, sample size, sampling and calibration of frequently used laboratory instruments, Chemical analysis based on various techniques of spectrometry, chromatography etc., Quality control and quality assurance in chemical Industries.

UNIT IV

General approach to method development and validation, Study of validation parameters, Accuracy, Precision, Linearity, Range, Limit of detection(LOD), Limit of quantification(LOQ), System suitability, Stability, Ruggedness, Robustness

Introduction, fundamentals, principle, instrumentation and applications of gas chromatography, HPLC and other hyphenated Techniques: LC-MS, GC-MS, IC-MS, HP-TLC, and ICP-MS Spectroscopy

Separation Methods: Theory and applications of separation methods in analytical chemistry: solvent extraction, ion exchangers including liquid ion exchangers.

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UNIT V

Literature Searching: On-line searching, Database, Scifinder, Scopus, CA on CD, Locating research article, Citation Index, Impact Factor

Writing scientific report: Planning, preparation, draft, revision and refining; writing project proposal to funding agency, Paper writing for International Journals, submitting to editors. Conference presentation, preparation of effective slides and presentation.

Introduction to Internet and its applications: web browsers – World Wide Web, Search Engines, e-journals, literature Survey in Chemistry, Popular journals and websites in Chemistry, Databases in Chemistry, literature searching and collection using e-journals.

Preparing presentations: i) Research papers : Using word processing software – MS Word/Latex/others, Drawing graphs and diagrams – Origin/Xmgrace/Excel/others. ii) Seminar presentations – Power point for oral and poster presentations

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Paper II: Analytical Biochemistry

Unit I

Fluorescence techniques- Basis, principle and procedure of fluorescence process, methods used - instruments, fluorophores, photobleaching, multicolour labeling, radiometric measurements, signal amplification, multi-photon excitation, external factors and fluorophore - fluorophore interactions. Applications of fluorescence technique.

Immunoanalytical methods- structure and characteristics of antibodies, polyclonal and monoclonal antibodies, antigens, immunoassays, immunofluorescent analysis by flow cytometry, immunofluorescence studies using confocal laser scanning microscopy.

Unit II

Biosensors- principle, types - electrode based biosensors, nano column and nano well biosensor, amperometric biosensor, potentiometric biosensor, optical, fibre optic and waveguide devices, Piezoelectric and surface acoustic wave biosensors, sensors in bioprocess control, flow injection analysis.

Biochips- what are biochips, protein and DNA biochips, methods for preparation of biochips and results generated by protein and DNA biochips.

Bioinformatics Biological data bases - DNA sequence data bases & protein sequence data bases SRS- Similarity searching BLAST, FAST A Multiple sequence alignment- Phylogeny. Structure database- Secondary structure prediction, Choufeat passman, Neural network methods. Predicting 3 dimensional folds (Threading).

Unit III

Laws of absorption and absorption spectrum. Principle, instrumentation and applications of UV, visible spectrophotometer and spectrofluorimetry. Atomic spectroscopy- principle and applications of atomic flame and flameless spectrophotometer. Basic principles and application of mass spectrometry. Basic principles and applications of NMR, ESR, ESI-MS, GC MS, CD- dichroism, X-ray diffraction, etc. Atomic Force Microscopy- instrumentation, its resolution and vibration, techniques involved, surfaces and biomolecules imaged by AFM.

Unit IV

Isolation of Biomolecules and cell organelles: Centrifugation: Basic principles of sedimentation, Sedimentation coefficient, Svedberg Unit. Various types of centrifuges and their uses. Rotors - Fixed angle, Vertical, Swing out, Zonal rotors. Preparative centrifugation-Differential density gradient centrifugation and centrifugal elutriation. Materials for preparation of density gradient. Analytical ultracentrifugation- determination of molecular weight and purity of biomolecules by analytical ultra centrifugation.

Chromatography - principle, instrumentation and application of column chromatography, ion exchange chromatography, Gel filtration, thin layer chromatography, affinity chromatography. Gas chromatography. HPLC, FPLC. General principle of electrophoresis . native and SDS PAGE, gradient gels, isoelectric focusing, 2D PAGE, membrane blotting of protein detection and recovery. Agarose gel electrophoresis, DNA sequencing gel, membrane blotting of nucleic acid, Hybridization of DNA dot blot and fluorescent in situ hybridization FISH, RFLP. PCR, RT-PCR and applications: Yield quantification analysis.

Unit V

Supramolecular chemistry- Properties of covalent bonds-bond length, inter-bond angles, force constant, bond and molecular dipole moments. Molecular and bond polarizability, bond dissociation enthalpy, entropy. intermolecular forces, hydrophobic effects. Electrostatic, induction, dispersion and resonance

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energy, magnetic interactions, magnitude of interaction energy, forces between macroscopic bodies, medium effects. Hydrogen bond.

Nano clusters and colloids in bioanalysis- general methods- colloidal synthesis, silanization, adsorption of polymers, SERS, DNA-coated nano-particles, surface enhanced adsorption, protein confirmation biochips, surface enhanced fluorescence.

Smart Materials and Nanomedicine- Calixarenes , Catenanes, Rotaxanes, Drug Encapsulation ,functional drug carriers, Smart materials, smart instruments, surgical robots, Tissue Regeneration Scaffolds, Imaging devices.

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Unit-I

Introduction to enzymes: What are enzymes, brief history of enzymes, nomenclature and classification of enzymes. Chemical nature of Enzymes. The basis of protein structure, the determination of primary structure, determination of protein structure by X-ray crystallography.

Specificity of Enzymes: Types of specificity, the Koshland "induced fit" hypothesis, Strain or transition – state stabilization hypothesis. Methods for isolation, purification and characterization of enzymes.

Immobilization of enzymes - principle, types and their applications, Biosensors and their applications, ELISA, EMIT.

Unit -II

Enzyme Catalysis and Kinetics: Factors affecting the rate of chemical reactions, kinetics of single substrate enzyme-catalyzed reaction, multi enzyme catalyzed reaction, and kinetics of inhibition of enzyme activity.

The Investigation of Active Site Structure and Chemical nature of Enzyme

Catalysis: The identification of binding sites and catalytic site, three dimensional structure of active site, mechanism of catalysis, mechanism of reaction catalyzed by enzyme without cofactors, metal-activated enzyme and metalloenzyme, coenzymes in enzyme catalyzed reactions.

Unit III

Enzymes in pharmaceutical industry, leather industry, detergent industry, food industry, research, therapeutic and clinical diagnosis. Use of enzymes in various industries - wool, dairy, juice & beverage, sucrose industry and use in medicine. Enzymes as thrombolytic agents, anti-inflammatory agents, debriding agents and digestive aids.

Unit IV

Enzyme and protein engineering: General introduction to protein engineering, Site-specific and multiple amino acid substitutions. Functional and structural consequences and limitations Strategies and approaches. Application of molecular modeling and structure predictions to protein engineering Molecular modeling Molecular mechanical calculations and geometry optimization Overview of current methods in prediction of secondary and tertiary structure from sequence.

Unit V

Enzyme Engineering- Enzyme reactors, objective, principle of enzyme engineering, steps involved, application of enzyme engineering, Abzymes and their applications. Ribozymes, bi- and poly functional enzymes, synzymes, monomeric and oligomeric enzymes, instrumental techniques used in enzymatic analysis, engineering with unnatural amino acid analogues. Site-specific incorporation of amino acid analogs by in vitro methods. De novo protein design & artificial proteins: Approaches used in designing and constructing novel proteins. Use of scaffold proteins.

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Unit I

DNA and basics of Recombinant technology- Basics Concepts DNA Structure and properties; Packing of DNA, supercoiled DNA, nucleosome, Inverted repeats, repetitive DNA sequence, satellite DNA. Restriction Enzymes- their recognition sequences and cleavage patterns. Steps of gene cloning- cDNA and genomic library, chemical synthesis of gene Hybridization techniques: Northern, Southern and Colony hybridization, Fluorescence in situ hybridization; Chromatin methyl interference assay. Labeling of DNA: Nick translation, random priming, probes.

Unit II

Tools in genetic engineering- Various expression vectors in bacteria and eukaryotes viz. Plasmid, bacteriophage, cosmid, Phasmid, shuttle, yeast vectors (ARS, Mini Chromosome and artificial vectors), Animal Virus derived vectors- SV-40 vector, Bovine papillomavirus & retroviral vectors. Integration of DNA insert into the vector, introduction into the suitable host, selection of recombinant clones. Multiplication, expression and integration of DNA insert in host genome. Insertion of Foreign DNA into Host Cells: Transformation, Transfection: Chemical and physical methods, liposomes, microinjection, macroinjection, electroporation, biolistics, somatic cell fusion.

Unit III

Analytical techniques- Restriction maps and mapping techniques, DNA fingerprinting, chromosome walking & chromosome jumping, DNA-Protein interactions: Electro mobility shift assay, DNase I footprinting, methyl interference assay. PCR - procedure of gene amplification, types of PCR and its applications- PCR in molecular diagnostics, viral and bacterial detection, PCR based mutagenesis. DNA sequencing- Maxam-Gilbert method, Sanger's Dideoxy chain termination method, Enzymatic DNA sequencing, RNA sequencing Human genome sequencing: Genetic and physical mapping techniques, sequencing strategies, and gene annotation. Chemical Synthesis of oligonucleotides. Gene silencing techniques- principle and application of gene silencing.

Unit IV

Transgenics- Transgenic plants- Gene constructs, vectors for production, transformation techniques; integration, inheritance, analysis and confirmation of transgene integration, various useful gene transfers. Problems in gene transfer, safety and regulations for transgenic plants. Applications and future prospects of transgenic plants.

Transgenic animals- Gene constructs, vectors used, transfection methods, embryonic stem cell transfer, targeted gene transfer; integration, detection and analysis of transgene integration. examples of transgenic animals.

Unit V

Applications of genetic engineering in medicine and health care- disease prevention- Genetically engineered biotherapeutics and vaccines and their manufacturing; disease diagnosis; disease treatment- interferons, monoclonal antibodies, drug designing, drug delivery and targeting; Gene therapy -principle, types and applications. Genetic engineering involved in forensic medicine.

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Text / Reference Books:

- The Tools of Biochemistry - Terrace E. Cooper (John Wiley)
- A biologist's Guide to Principles and techniques of Practical Biochemistry- Ed Bryan, L. Williams & Keith Wilson (Edward Arnold).
- Biochemical Research Techniques - A Practical Introduction - Ed. John M. Wriggelsworth.
- Principles & Techniques of practical Biochemistry- Keith Wilson & John Walker.
- Analytical Biochemistry - David J. Home & Hazelpeck (Longman).
- Varley's Practical Clinical Biochemistry (5th Edition, A.H. Cowenlock & M. Bell (Heinemann).
- A. Manual of Radiobiology - J.C. Steward & D.M. Hawcroft (Sidgwick & Jackson).
- Instrumental Methods of Chemical Analysis - B.K. Sharma (Goel Publications).
- Text of Books of Biochemistry - West and Todd.
- Biophysical Chemistry - D. Freifelder.
- Biophysical Chemistry - Upadhyaya and Nath (Himalayan Publications).
- Molecular Cloning by Sambrook et al, Cold Spring Harbor Laboratory, New York.
- Lehninger's Principles of Biochemistry- Nelson, David L and C
- Lehninger, Principles of Biochemistry, Kalyani Publishers.
- T. Palmer, Prentice Hall, Understanding Enzymes. Horwood Publishing Series
- Stryer, L. Biochemistry. W. H. Freeman & Co.
- Voet, D. and Voet, J.G., Biochemistry, John Wiley & Sons.
- Haefner, Modeling Biological Systems, Springer Publ.
- Fundamentals of Enzymology by Price and Stevens (2002): Oxford University Press.
- Enzyme Technology by Helmut Hülling (1998): John Wiley
- Introduction to Proteins Structure by Branden and Tooze (1998): Garland Publishing Group.
- Sambrook J, Fritsch E. F. and Maniatis (1989) Molecular cloning, vol. I, II, III, II nd edition, Cold spring harbor laboratory press, New York.
- DNA Cloning : A practical approach D.M. Glover and D.B. Hames, RLP, Oxford, 1995
- Molecular and cellular methods in Biology and Medicine, P.B. Kaufman, W. Wu , D. Kim and L.J. Cseke, CRC Press Florida 1995
- Methods in Enzymology Guide to Molecular Cloning Techniques, Vol. 152
- S.L. Berger and A. R. Kimmel, Academic Press Inc, San Diego, 1996
- Methods in Enzymology Gene Expression Technology, Vol. 185D.
- V. Goedel, Academic Press Inc, San Diego, 1990
- DNA Science: A First Course in Recombinant Technology, D. A. Mickliss and G. A. Freyer, Cold Spring Harbor Laboratory Press, New York, 1990
- Molecular Biotechnology, 2nd Ed. S. B. Primrose, Blackwell Scientific publishers, Oxford, 1994
- Milestones in Biotechnology, Classic Papers on Genetic Engineering, J. A. Davis and W. S. Reznikoff, Butterworth-Heinemann Boston 1992
- Route Maps in Gene Technology, M. R. Walker, and R. Rapley, Blackwell Science, Oxford, 1997
- Genetic Engineering : An Introduction to Gene Analysis and Exploitation in Eukaryotes, S. M. Kingsman, Blackwell Scientific Publications, Oxford, 1998
- Primrose, Principles of Genome analysis, Oxford Univ. Press.
- Old & Primrose., Principles of gene manipulation, 5th Edn. S.B. Blackwell Scientific Pubs.
- Watson, Recombinant DNA, Freeman & Co
- Glick & Pasternak, Molecular Biotechnology, Panama Pubs.
- Smith, J.E. 1996. Biotechnology, Cambridge Univ. Press.
- Benjamin Lewin, Genes VII, Oxford Univ. Press.
- Enderle Blanchard & Bronzino, Introduction to Biomedical Engineering Elsevier Publ.
- Arthur M. Lesk, Introduction to Bioinformatics, Oxford University Press,
- Baxevanis and B.F. Ouellette. Bioinformatics: A practical Guide to the Analysis of Genes and Proteins, Third edition, Wiley-Interscience.
- D.W. Mount, Bioinformatics - Sequence and Genome Analysis, 2nd edition, CBS publishers.
- D. Higgins and W. Taylor (Eds), Bioinformatics- Sequence, structure and databanks, Oxford University Press.
- M. Campbell & L. J. Heyer, Discovering Genomics, Proteomics & Bioinformatics, Pearson Education.

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- S.R. Pennington & M.J. Dunn, Proteomics : from protein sequence to function, Viva Books Pvt. Ltd.,
- Anderson, Durston, Polle (1970) Thesis and assignment writing Wil Eastern Limited
- Beveridge, B. (1979) The Art of Scientific investigation, W.E. Norton. & Co. New York
- Biostatistical analysis . J.H. Zar, 4th edition. Pearson Education, Inc. India.
- Braun, R.P. Introduction to instrumental analysis, McGrawHill.
- Wilson & Walker, Principles and Techniques of Biochemistry and Molecular Biology . 6th Edn, Cambridge Univ. Press.
- Practical Skills in Chemistry, J. R. Dean, A. M. Jones, D. Holmes, R. Reed, J. Weyers and A Jones, Pearson Education Ltd. [Prentice Hall] (2002)
- OSU safety Manual 1.01.
- Research Methodology. Methods and Techniques : C. R. Kothari,
- Tests, Measurements and Research Methods in Behavioural Sciences : A. K. Singh
- Physical Method in Chemistry, R.S. Drago, Saunders College Publishing.
- Computers and Common Sense R. *Hunt and Shelley*, Prentice Hall, New Delhi (1998)
- Computer Programming in Fortran-90 V. *Rajaraman*, Prentice Hall, New Delhi (1990)
- Computer and Chemistry: introduction to programming and numerical methods T. *R. Dickson*, Freeman (1968)
- Computer programs for chemistry *D. F. Detar* W. A. Benjamin Inc, New York Vol. 1-3 (1968-69)