

Contents

<i>Preface</i>	v	Selection of a Suitable Density Gradient Medium	21
1. RESEARCH METHODOLOGY 1—12		Types of Density Gradient Media	22
Introduction	1	Collection of Fractions	22
What is Research?	1	Measurement of Gradient Density	22
Research – Characteristics	2	Sample Application to the Gradient	22
Research – Purpose, Aims & Objectives	3	Recovery and Monitoring of Gradients	23
Research – Motivation	3	Analysis of sub-cellular Fractions	24
What is Methodology?	4	Homogeneity Assessment	24
Research Methodology	4	Presentation of the Results	24
Research Methodology – Different Steps	5	Types of Centrifuges	24
Research Methodology – Importance	5	Rotor Types and Designs	26
Quality Research – What is it?	5	Centrifugation Technique	27
Types of Research	6	Reagents Required	27
Other Types of Research	8	Procedure	27
Basic Approaches to Research	9	Precautions to be taken during Centrifugation	28
What is Research Data?	9	Rotor Selection	28
Research Writing	10	Care of Rotors and Accessories	29
Reasons for Research	10	Centrifuge Containers	30
Writing a Paper or Dissertation	10	Selection of Centrifuge Tubes	30
Different Steps of Writing	11	Hazards met with during the Operation of Ultracentrifuge	31
Criteria of Good Research	12	What are Centrifuges used for?	31
Significance of Research	12	Other types of Centrifuges	31
2. CENTRIFUGATION 13—38		Safety Aspects in use of Centrifuges	36
Introduction	13	Applications of Centrifugation in Zoology	37
General Methods of Separation	13	Uses of Centrifuges	37
Centrifugation	14	3. ELECTROPHORESIS 39—78	
Early History of Centrifugation	14	Introduction	39
Definition of Centrifugation	15	Definition of Electrophoresis	39
Theoretical Basis of Centrifugation	15	Brief History of Electrophoresis	40
Basic Principle of Sedimentation	16	Basic Theory of Electrophoresis	41
The Centrifuge Machine	18		
Types of Centrifugation	19		

How Electrophoresis Works and Separation Occurs?	41	Principle	87
Method of Electrophoresis	42	AAS – Instrumentation	88
Factors that affect Electrophoresis	43	Flame Atomizers	88
Instrumentation for Electrophoresis	44	Electro-thermal Atomizers	89
Apparatus Electrophoresis	44	Sources of Radiation	89
Types of Electrophoresis	45	Spectrometer – Measurements	90
Techniques used in Biological Research	49	AAS – Other Techniques	91
Gel Electrophoresis	58	Uses of AAS	95
Sds-Page Technique	61	AAS – Applications	95
Sample Preparation of Reagents and Loading Buffers for SDS-PAGE	63	Applications of AAS – Summary of Different Techniques	96
Protein Gel Stains	63		
Polyacrylamide Gels	64	5. CHROMATOGRAPHY	98—122
Precast Gels	65	Introduction	98
Agarose Gel Electrophoresis (AGE)	65	Definition of Chromatography	98
Agarose vs. Polyacrylamide	68	Brief History of Chromatography	99
Starch	68	Chromatography – Types and Techniques	100
Agarose Gel Electrophoresis – General Protocol	68	Planar Chromatography	100
Background of the Process	68	Column Chromatography	101
Objective of the Experiment	69	Liquid Chromatography (LC)	102
Materials Required	69	Ultra-Performance Liquid Chromatography (UHPLC or UPLC Technology)	102
Recipes - Preparation, Properties and Uses	69	Fast Protein Liquid Chromatography (FPLC)	103
Preparation of the Gel	69	Supercritical Fluid Chromatography (SFC)	104
Loading the Gel	70	Supercritical Fluid Extraction (SFE)	104
Running the Gel	70	Paramagnetic Method	106
Staining the Gel	70	Paper Chromatography	106
Detection Methods – Blotting Techniques	71	– General Protocol	
Other Methods	77	Thin Layer Chromatography (TLC)	108
Applications of Electrophoresis	77	What TLC can offer today?	108
		Preparation of the Plate	109
4. SPECTROPHOTOMETRY	79—97	Preparation of the Sample	109
Introduction	79	Technique of TLC	109
Spectrophotometry	80	Principle and Process of Separation	110
Spectrophotometer	80	Analysis of the Results	111
Beer–Lambert's Law	82	Isolation of the Compounds	111
Spectrophotometric Analysis – General Approach	82	Gas Liquid Chromatography (GLC)	111
Applications of Spectrophotometry	85	Gas Solid Chromatography (GSC)	112
Atomic Absorption Spectroscopy (AAS)	85	Carrier Gas	112
Definition	85	Sample Injection System	112
History	86	Direct Injection with a Syringe	112
		Injection with a Valve/Sample Loop/Loop Injection	113

Gas Liquid Chromatography (GLC)	113	Histochemical Techniques	147
Injection of the Sample	114	Fixation	147
How the Column works?	114	Types of Fixation	148
The Packing Material	115	Chemical Fixation	149
Temperature of the Column	115	Types of Chemical Fixatives	149
How Separation works on the Column?	115	Factors that affect Fixation	150
Retention Time	116	Fixing the Tissue	151
The Detector	116	Washing the Tissue	152
Flame Ionization Detector	116	Chemical Fixation with Formaldehyde/ Other Chemicals	152
High Performance Liquid Chromatography (HPLC)	117	Frozen Section Fixation	152
HPLC Instrument	118	Method of Fixation	152
HPLC – Operation of the Instrument	119	Processing – Dehydration, Clearing and Infiltration	153
Types of Columns	119	Dehydration	153
Common Mobile Phases	119	Embedding	153
How Does HPLC Work?	120	Paraffin	153
HPLC – Types	121	Plastic	154
HPLC – Applications	121	Sectioning	154
Chromatography – Applications	122	Ultramicrotome	155
6. POLYMERASE CHAIN REACTION (PCR)	123—144	Cryostat	155
Introduction	123	Staining of Fixed Material	156
PCR – What it is?	124	Methods of Staining	156
PCR – Principle	124	Progressive Staining vs. Regressive Staining	156
PCR – General Protocol	124	H & E or HE Staining	157
Materials Required	124	Protocol	157
Method	124	Single Staining	157
Recipes	125	Double Staining	158
Precautions to be taken	126	Common Laboratory Stains	158
Three Main Steps of PCR	126	Basic and Acid Dyes	159
Results after running a PCR	129	Artifacts	160
PCR - Important Points to Remember	131	Pre-histology artifacts	160
Limitations of PCR	131	Post-histology artifacts	160
Different Schemes and types of PCR	132	Staining Methods	160
Applications of PCR in different Disciplines	141	Direct Staining (Simple Staining)	160
PCR –Benefits, Drawbacks and Limitations	143	Indirect Staining (Negative Staining)	161
Conclusion	144	Differential Staining Technique	161
7. CHEMICAL ANALYSIS OF ANIMAL TISSUES	145—183	Gram Staining Technique (Gram's Method)	161
Histology	145	Gram's Staining – Procedure	162
Histology – History	145	Gram Staining - Limitations	163
Histochemistry	146	Gram's Stain – Modifications	163
		Gram Staining – Applications	163
		Progressive Staining	164

8. ANIMAL CULTURE TECHNIQUES

Sterile Work Area	196	Resuscitation of Frozen Cell Lines	214
Incubation Facilities	196	Sub-culture of Adherent (Monolayer)	
Refrigerators and Freezer (-20°C)	197	Cell Lines	214
Microscopes	197	9. WATER POLLUTION	217—248
Tissue Culture Ware	197	Introduction	217
Facilities for Washing up and		Definition	218
Sterilizing	198	Water Quality	218
Liquid Nitrogen/Liquid Freezer	198	Common Water Pollutants	218
Water Still or Reverse Osmosis		Dirt	218
Apparatus	198	Bacteria	219
Filter sterilization	199	Nutrients	219
Facilities for Counting Cells	199	Water Monitoring - Importance	219
General Small Items of Equipment	199	Measuring Water Quality - Parameters	219
Culture Media	199	Temperature	219
Basic Media	200	Dissolved Oxygen (DO)	220
Buffering Capacity	200	pH	220
Glutamine and Amino acids	200	Turbidity	220
Serum	201	Water for Human Consumption	220
Antibiotics and Antimycotics	201	Water for Industrial and Domestic Use	221
Supply and Preparation of the		Environmental Water Quality/Ambient	
Culture Media	201	Water Quality	221
Culturing Animal Cells - Selecting		Quality Water Monitoring	221
Sources of Tissue	202	Monitoring Strategy	222
Adult or Embryonic Tissue	202	Environmental Impact Assessment	222
Embryonic Stem Cells	202	Drinking Water Indicators	222
Normal or Neoplastic Tissue	202	Alkalinity of water	223
Selecting types of Animal Cell Culture	203	CDOM - Coloured or Chromophoric	
Organ Culture or Cell Culture	203	Dissolved Organic Matter	223
Adherent or Suspension Culture	203	Dissolved Organic Matter	
Primary Cultures or Continuous		(DOM/CDOM/FDOM)	224
Cell Lines	203	DOC - Dissolved Organic Carbon	
Primary Cultures: Advantages and		(DOC)	225
Limitations	204	Total Carbon, Total Organic Carbon and	
Continuous (Clonal) Cell Lines:		Total Inorganic Carbon	226
Advantages and Limitations	205	Total Carbon (TC)	226
Types of Media used in Animal Cell		Total Organic Carbon (TOC)	227
Culture	206	Total Inorganic Carbon /Dissolved	
Media and the Serum	210	Inorganic Carbon (TIC or DIC)	229
Requirement of Rich Media for Culture	211	Environmental Indicators	229
Do's and Don'ts of Cell Culture		Physical Indicators	229
(Basic Techniques)	211	Transparency (Turbidity)	231
Animal Cell Culture - Applications	213	Chemical indicators	231
Animal Cell Culture - General Protocol	213	Biological Indicators	234
Aseptic Technique and Good Cell		Microbiological Testing/Analysis	234
Culture Practice	213	Biological Monitoring	235
General Procedure	213		

Toxicology	236	Projects which need EC/EIA	252
Toxicity - Characterization	237	EIA - Follow-up	252
Assessment of Risk	238	EIA Around the world	253
Environmental Toxicology	238	Environmental Assessment (EA)	253
Aquatic Toxicology	239	EIA - Content	253
Toxicity - Measurement	240	EIA - Structure	254
Toxicology - Terminology	240	EIA - Procedure	254
Acute Exposure	240	Environmental Impact Statement (EIS)	254
Chronic Exposure	240	Trans-boundary Application	254
Toxicity and Hazard	241	EIA in India	255
LD50 and LC50	241	EPA in India	255
Routes of Exposure	241	Amendments to the EPA, Rules, 1986	256
LC50	242	EPA, 1986 - Important Definitions	256
LD50	242	Environmental Appraisal Committees	258
Carrying out the tests (LD50 and LC50)	243	Environmental Appraisal Procedure	258
Importance of LD50 for occupational health and safety	243	Environmental Appraisal Monitoring	258
Uses of LD50	243	Coastal Area Management (CAM)	259
Can animal LD50 data be applied to humans?	244	Island Development Authority (IDA)	259
Source of LD50 and LC50 values	244	Studies on Carrying Capacity	259
Bioaccumulation/Bio-Concentration	244		
Definition	244	11. BIOSTATISTICS AND ANIMAL RESEARCH	261—286
Related Definitions	245	Introduction - Statistics and Biostatistics	261
Bio-concentration factor (BCF)	245	Definition of Statistics and Biostatistics	262
Bioaccumulation Factor (BAF)	246	Differences between Biostatistics and Statistics	262
Bio- or Biological Magnification/ Bio-Amplification	246	Biostatistics - What is it?	263
Bioaccumulation versus Bio-magnification	247	Brief History of Biostatistics	263
		Role of Statistics in Biology	264
		Importance of Mathematics in Biology and Areas of Research	264
		Importance of Statistical Analysis	265
		Common Factors shared by Botanists and Zoologists	265
		Statistical Applications in Biological Research	266
		Averages	266
		Statistical Averages	268
		Natural Progression	272
		Standard Deviation (SD)	272
		Estimation of SD	273
		Correlation Coefficient	274
		Standard Error of Mean (SEM) and its Applications	274
		SD and SE	274
10. ENVIRONMENTAL IMPACT ASSESSMENT	249—260		
Introduction	249		
EIA - History	249		
EIA - Purpose	250		
EIA - Legal Sanctity	250		
EIA - Different Steps	250		
Identification	251		
Prediction	251		
Evaluation	251		
EIA - Assessment Methods	251		
Eia - Projects and Process of Clearance	251		

Confidence Interval (CI) or Fiducial Limits	275	Advantages and Disadvantages	303
Standard Error	275	Third Generation: Integrated Circuits (1964 – 1975)	303
SE of the mean versus SD	276	Advantages and Disadvantages	303
Relative Standard Error	276	Fourth Generation: Microprocessor (1975 to the present)	304
Analysis of Variance	276	Advantages and Disadvantages	304
ANOVA – Characteristics	276	Fifth Generation: Artificial Intelligence (Present and beyond)	305
ANOVA – Logic	277	Computer Applications in Animal Science	305
Types of ANOVA	277	Methods in Zoology	305
MANOVA (Multivariate Analysis of Variance)	278	Use of Computers	306
Factorial ANOVA	278	Applied Zoology	306
Balanced ANOVA	278	Using the Web for Research	307
Three-way ANOVA	279	Variety of Tools used by Zoologists	308
Between-Subjects ANOVA	279	Animal Traps	308
Within-Subjects ANOVA	279	Collection Containers	309
Mixed-Model ANOVA	279	Tracking Devices	309
ANCOVA (Analysis of Covariance)	279	Computer Software	310
Regression	279		
Correlation Coefficient	281	13. WORD PROCESSING WITH A COMPUTER	311–318
Chi-square Test	281	Introduction	311
Applications of Chi-square Test	281	Essential Functions of Word Processing	312
Student's <i>t</i> Test	282	Text Input	312
Application of Paired and Unpaired <i>t</i> Test	282	Text Manipulation	312
Statistical Software	284	Text Formatting	313
Modern Biostatistics	285	Text Output	313
Role of Statistics in Biology and Medicine in Developing Countries	285	Advanced Features of Text Processing	314
Applications of Biostatistics	285	Styles	314
Use of Statistics in Daily Life	286	Macros and Merging	314
		Version Control	314
12. COMPUTER APPLICATIONS IN ANIMAL SCIENCE	287–310	Automatic References and Indexes	314
History of Computers — Timeline of Evolution	287	Desktop Publishing Capabilities	314
Classification of Computers	298	Computer Software for Word Processing	315
Classification of Computers by Size and Power	299	Working with the Text	316
Five Generations of Computers	301	Word Processing	316
First Generation: Vacuum Tubes (1942 – 1955)	301	Word Processing Software	316
Advantages and Disadvantages	302	Presentation Programme	317
Second Generation: Transistors (1955 – 1964)	302	Word Processing - Advantages and Disadvantages	317

14. WORD PROCESSING SOFTWARE**319—337**

Introduction	319
Microsoft Word	319
MS Word - Early History	320
Word for Windows	321
Word for MAC	321
File Formats	321
File Extensions	322
Binary Formats (Word 97 - 2007)	322
XML Document (Word 2003)	322
Microsoft Office XML Formats	322
Limitations and Differences with Open Office XML	323
Cross-Version Compatibility	323
Third-Party Formats	323
Image Formats	324
Features	324
Templates	324
Word Art	324
Macros	324
Layout Issues	325
Bullets and Numbering	325
Auto Summarize	325
Password Protection	325
Flaws: Reception	326
Differences between Word Processing, Desktop Publishing and Presentation Software	326
Microsoft Powerpoint	327
MS Powerpoint - History	327
PowerPoint - What is?	328
Powerpoint Views, Slide Master and Templates	328
Creation of a Master Slide	329
Powerpoint Viewer	329
Powerpoint Viewer File Formats	330
Microsoft Excel (Ms Excel)	330
Basic Operation of Excel	330
Macro Programming - VBA Programming	331
History of Microsoft Excel	331
Charts supported by Excel	332
Excel - Data Storage and Communication	332

Number of Rows and Columns	332
File Formats	332
XML Spreadsheet	332
New and Old Excel 2007 Formats	332
Use of other Window Applications	333
Use of External Data	333
Export and Migration of Spreadsheets	333
Password Protection	333
Microsoft Excel Viewer	334
Errors (Quirks)	334
Statistical Functions	334
Excel MOD Function Error	334
Date Problems	334
File Names	334
Numeric Precision	335
Different Versions of Excel	335
Impact	336
Security	336
Basic Skills of Excel	337
Navigation - Getting Started	337
Quick Access Toolbar	337
Ribbon	337
Formula Bar	337
Spreadsheet Grid	337
Status Bar	337

15. INTERNET CONCEPTS 338—349

Internet - What it is?	338
Basic Concepts of Internet	339
IP Address	339
Domains and Sub-domains	339
Servers	340
Network - What it is?	340
Local Area Networks (LANs)	340
Wide Area Networks (WANs)	340
Combinations of Networks	340
The Internet	341
Basics of the Internet	341
Basic Functions and Tools of the Internet	341
Logging In on to a Local System	342
World Wide Web (WWW or W3)	343
- What it is?	343
WWW - Basic Concepts	343
Browser	343

Hypertext and Hypermedia	343	Origin of Email	355
Hypertext Markup Language (HTML)	344	How to Send and Receive Email?	355
Uniform Resource Locator (URL)	344	Email Online	355
Hypertext Transport Protocol (HTTP)	344	How to Write an Email?	355
Homepage	344	Email - Structure	356
Clients and Servers	344	Concept of a Header	356
Internet Access Tools	344	Using Email	357
Internet and its Usage	344	Email Fields	357
Features of Internet	345	Valid Email Address	358
Geographic Sharing	345	Email - Rules for Proper Use	358
Architecture	345	When Composing and Sending a Message	358
Universal Access	345	When Receiving a Message	359
Internet - Benefits	345	Important Criteria to the Success of Email	360
Access to the Internet	345	How Email Works?	
Privacy Issues	346	(MTA, MDA, MUA)	360
Privacy	346		
Web Searching	346	17. FILE TRANSFER PROTOCOL (FTP)	361—366
Web Site - What it is?	346	Introduction	361
Web Address	346	Objectives of FTP	361
Uses of internet	346	The FTP Server - History	362
Advantages of Internet	347	FTP - How does it work?	362
Disadvantages of Internet	347	The FTP - Model	363
The Internet and Education	348	Mode of File Transfer	364
The Internet and Culture	349	Passive FTP	364
The Internet and Personal Relationships	349	Further FTP Security	364
The Internet and our Daily Life	349	Secure File Transfer (SFT)	365
Internet in different fields	349	SFT - Features and benefits	365
		How to use FTP?	366
16. ELECTRONIC MAIL (E-mail or Email)	350—360	Appendix 1	367—373
Introduction	350	Appendix 2	374—378
History of Email	350	Appendix 3	379—387
Email - Time line of Evolution	351	Appendix 4	388—399
Email Software Platforms	354	References	400—404
Email - Spelling Options	354		