

Contents

1. THE THEORY AND PRACTICE OF CENTRIFUGATION	1
D. Rickwood	
Introduction	1
Theory of Centrifugation	1
Sedimentation theory	1
Non-ideality of biological particles	2
Sedimentation coefficients	3
Practical calculations of centrifugal force and centrifugation times	3
Types of Centrifugal Separations	5
Differential pelleting	5
Rate-zonal centrifugation	6
Isopycnic centrifugation	7
Centrifuges and Associated Equipment	7
Types of centrifuge	7
Drive systems of centrifuges	10
Centrifuge rotors	12
Centrifuge tubes, bottles and caps	22
Centrifuge safety	25
Centrifugation Media	27
Introduction	27
Properties of nonionic gradient media	28
Properties of ionic gradient media	38
Choice of Gradient Medium and Centrifugation Conditions	41
References	42
2. CHOICE OF CONDITIONS FOR DENSITY GRADIENT CENTRIFUGATION	45
B.D. Hames	
Introduction	45
Choice of Rotor	46
Introduction	46
Comparison of rotors for rate-zonal centrifugation	47
Comparison of rotors for isopycnic centrifugation	50
Choice of Density Gradient for Rate-zonal Centrifugation	50
Introduction	50
Choice of gradient solute and solvent	51
Choice of gradient shape	51
Choice of Density Gradient for Isopycnic Centrifugation	55
Choice of gradient solute and solvent	55
Choice of gradient shape	55

Formation of Gradients	61
Preparation of gradients for rate-zonal centrifugation	61
Preparation of gradients for isopycnic centrifugation	71
Centrifugation of Gradients	73
Rate-zonal centrifugation	73
Isopycnic centrifugation	78
Fractionation of Gradients	82
Introduction	82
Unloading gradients from the bottom	82
Unloading gradients from the top	84
Direct recovery of bands	86
Analysis of Gradients	87
Determination of the gradient profile	87
Analysis of samples	88
References	90
Appendix: A Computer Program for the Calculation of Equilibration Times and Profiles of Self-forming Gradients	91
3. CENTRIFUGAL METHODS FOR CHARACTERISING MACROMOLECULES AND THEIR INTERACTIONS	95
D. Rickwood and J.A.A. Chambers	
Introduction	95
Precautions Required when Fractionating Macromolecules	95
Rate-zonal Separations of Macromolecules	96
Introduction	96
Choice of gradient medium and gradient shape	96
Choice of rotor and centrifugation conditions	97
Experimental Procedures Used for Rate-zonal Separations	98
Separations on non-denaturing gradients	98
Separations on denaturing gradients	100
Use of rate-zonal centrifugation to study the interactions of macromolecules	102
Isopycnic Separations of Macromolecules	106
Introduction	106
Factors affecting the density of macromolecules in solution	106
Choice of gradient medium and shape	107
Preparation of gradient media and reagents	108
Choice of rotors and centrifugation conditions	108
Experimental Procedures Used for Isopycnic Separations	109
Fractionation of DNA on the basis of base composition	110
Fractionations of different conformations of DNA	112
Separation of single and double-stranded DNA and RNA-DNA hybrids	114
Fractionation of RNA	116
Fractionations of proteins, lipoproteins and proteoglycans	116

Fractionations of density-labelled macromolecules	117
Separation of proteins, DNA and RNA	118
Isolation of nucleoprotein complexes	119
Use of isopycnic centrifugation to study interactions of macromolecules	122
References	125
4. MEASUREMENT OF SEDIMENTATION COEFFICIENTS AND COMPUTER SIMULATION OF RATE-ZONAL SEPARATIONS	127
B.D. Young	
Introduction	127
Sedimentation Coefficients	128
Methods of Measuring Sedimentation Coefficients	129
Introduction	129
Isokinetic gradients	129
Pre-computed tables	130
Computer programs	132
Simulation Techniques	138
Troubleshooting	139
References	140
Appendix A: Pre-computed Tables for Calculating Sedimentation Coefficients	141
Appendix B: A Program for the Calculation of Sedimentation Coefficients in Swing-out Rotors	151
Appendix C: A Program for the Calculation of Sedimentation Coefficients in Vertical Rotors	155
5. ISOLATION OF SUBCELLULAR ORGANELLES AND MEMBRANES	161
J. Graham	
Introduction	161
Homogenisation	162
Homogenisers	162
Homogenisation media	164
Homogenisation of rat liver	165
Homogenisation of tissue culture cells	166
Differential Centrifugation	167
Introduction	167
Fractionation of rat-liver homogenates	168
Analysis of fractions	168
Partial purification of fractions	169
Simple Sucrose Density Barrier Methods	171
Separation of rough and smooth microsomes from fraction S3	171

Purification of nuclei	172
Purification of plasma membrane from pellet P1	173
Purification of mitochondria from pellet P1	173
Use of Discontinuous and Continuous Sucrose Gradients	174
Purification of lysosomes from pellet P3	174
Effect of gradient centrifugation on mitochondria	174
Isolation of Golgi membranes from pellets P2 and P3	175
Alternative Centrifugation Gradient Media	176
Permeability of membranes to gradient solutes	176
Osmotic effects of gradient media	176
Iodinated gradient media	177
Percoll gradients	178
Fractionation of Tissues Other than Rat Liver	180
Introduction	180
Isolation of plasma membrane from the P2 and P3 pellets of tissue culture cells	180
Isolation of plasma membrane from the P4 pellet of tissue culture cells	181
Acknowledgements	181
References	181
6. CENTRIFUGAL SEPARATIONS OF MAMMALIAN CELLS	183
A. Brouwer, R.J. Barelds and D.L. Knook	
Introduction	183
Use of Centrifugation for Cell Separations	183
Separation on the basis of density	185
Separation according to sedimentation rate	185
Gradients for cell separations	188
Problems and artifacts of cell separations	188
Characterisation of Cells and Analysis of Results	192
Guidelines for Devising a Method for Cell Separations	193
Experimental Protocols for Cell Separations	194
Isolation of rat-liver cells	194
Isopycnic centrifugation of cells	196
Velocity sedimentation of cells	202
Centrifugal elutriation of cells	202
Acknowledgements	213
References	213
Appendix A: Estimation of the Flow Rate and Rotor Speed Necessary to Separate Cells by Centrifugal Elutriation	214
Appendix B: Isolation of Liver Cells	217

7. SEPARATIONS IN ZONAL ROTORS	219
J. Graham	
Introduction	219
MSE BXIV and AXII Rotors	219
General design	219
Operation	224
Beckman Batch-type Zonal Rotors	226
Kontron Zonal System	227
General design	227
Operation	228
Reorienting Zonal Rotors	228
General design	228
Operation of the Sorvall TZ 28 rotor	229
Continuous-flow Rotors	230
Sorvall TZ 28 rotor	230
Beckman CF-32Ti and JCF-Z rotors	230
Practical considerations	232
Gradient Design	232
Examples of Separations Using Zonal Rotors	234
Separation of human blood cells	234
Fractionation of membranes from a rat-liver nuclear pellet	236
Fractionation of a tissue culture cell post-nuclear supernatant	238
Harvesting of virus from tissue culture fluid	240
Separation of 40S and 60S ribosomal subunits	243
Separation of the F and HN glycoproteins from Sendai virus	244
Ultracentrifugal analysis of immune complex formation between monoclonal antibodies and human IgG	246
Acknowledgements	248
References	248
8. ANALYTICAL ULTRACENTRIFUGATION	251
R. Eason	
Introduction	251
Sedimentation Velocity Analysis	252
Sedimentation coefficient of a pure, homogeneous sample	253
Molecular characterisation by sedimentation/diffusion	264
Active band centrifugation	269
Sedimentation analysis of interacting systems and polydisperse systems	271
Sedimentation Equilibrium Analysis	274
Molecular weight of a pure, homogeneous sample	274
Polydisperse and interacting macromolecular systems	279
Interaction of small molecules with macromolecules	281
Analysis of a multicomponent systems	282

Banding of DNA in self-generating density gradients	283
Analysis of purified, highly-charged molecules	285
Conclusions	285
References	286

APPENDICES

I. Nomogram for Computing Relative Centrifugal Force	287
II. Chemical Resistance Chart for Tubes and Zonal Rotors	289
III. Specifications of Ultracentrifuge rotors	293
IV. Equations Relating the Refractive Index to the Density of Solutions	305
V. Marker Enzymes and Chemical Assays for the Analysis of Subcellular Fractions	307
VI. Names and Addresses of Suppliers of Centrifuges and Ancillary Equipment	333
VII. Glossary of Terms	337
INDEX	345