

# Contents

<b>1</b>	<b>Absorption Spectroscopy Theory</b>	<b>1</b>
1.1	Introduction	1
1.2	Characteristics of an Absorption Spectrum	2
1.3	Beer–Lambert–Bouguer Law	4
1.4	Effect of the Environment on Absorption Spectra	6
	References	11
<b>2</b>	<b>Determination of the Calcofluor White Molar Extinction Coefficient Value in the Absence and Presence of <math>\alpha_1</math>-Acid Glycoprotein</b>	<b>13</b>
2.1	Introduction	13
2.2	Biological Material Used	13
2.2.1	Calcofluor White	13
2.2.2	$\alpha_1$ -Acid glycoprotein	13
2.3	Experiments	16
2.3.1	Absorption spectrum of Calcofluor free in PBS buffer	16
2.3.2	Determination of $\varepsilon$ value of Calcofluor White free in PBS buffer	16
2.3.3	Determination of Calcofluor White $\varepsilon$ value in the presence of $\alpha_1$ -acid glycoprotein	16
2.4	Solution	17
	References	19
<b>3</b>	<b>Determination of Kinetic Parameters of Lactate Dehydrogenase</b>	<b>21</b>
3.1	Objective of the Experiment	21
3.2	Absorption Spectrum of NADH	21
3.3	Absorption Spectrum of LDH	22
3.4	Enzymatic Activity of LDH	22
3.5	Kinetic Parameters	22
3.6	Data and Results	22
3.6.1	Determination of enzyme activity	23
3.6.2	Determination of kinetic parameters	23
3.7	Introduction to Kinetics and the Michaelis–Menten Equation	26

3.7.1	Definitions	26
3.7.2	Reaction rates	26
	References	32
<b>4</b>	<b>Hydrolysis of <i>p</i>-Nitrophenyl-<math>\beta</math>-D-Galactoside with <math>\beta</math>-Galactosidase from <i>E. coli</i></b>	<b>34</b>
4.1	Introduction	34
4.2	Solutions to be Prepared	35
4.3	First-day Experiments	35
4.3.1	Absorption spectrum of PNP	35
4.3.2	Absorption of PNP as a function of pH	36
4.3.3	Internal calibration of PNP	37
4.3.4	Determination of $\beta$ -galactosidase optimal pH	39
4.3.5	Determination of $\beta$ -galactosidase optimal temperature	40
4.4	Second-day Experiments	40
4.4.1	Kinetics of <i>p</i> -nitrophenyl- $\beta$ -D-galactoside hydrolysis with $\beta$ -galactosidase	40
4.4.2	Determination of the $\beta$ -galactosidase concentration in the test tube	42
4.5	Third-day Experiments	44
4.5.1	Determination of $K_m$ and $V_{max}$ of $\beta$ -galactosidase	44
4.5.2	Inhibition of hydrolysis kinetics of <i>p</i> -nitrophenyl- $\beta$ -D-galactoside	45
4.6	Fourth-day Experiments	47
4.6.1	Effect of guanidine chloride concentration on $\beta$ -galactosidase activity	47
4.6.2	OD variation with guanidine chloride	48
4.6.3	Mathematical derivation of $K_{eq}$	48
4.6.4	Definition of the standard Gibbs free energy, $\Delta G^\circ$	51
4.6.5	Relation between $\Delta G^\circ$ and $\Delta G'$	51
4.6.6	Relation between $\Delta G^\circ$ and $K_{eq}$	52
4.6.7	Effect of guanidine chloride on hydrolysis kinetics of <i>p</i> -nitrophenyl- $\beta$ -D-galactoside	56
	References	57
<b>5</b>	<b>Starch Hydrolysis by Amylase</b>	<b>59</b>
5.1	Objectives	59
5.2	Introduction	59
5.3	Materials	61
5.4	Procedures and Experiments	61
5.4.1	Preparation of a 20 g l <sup>-1</sup> starch solution	61
5.4.2	Calibration curve for starch concentration	61
5.4.3	Calibration curve for sugar concentration	63
5.4.4	Effect of pH	64
5.4.5	Temperature effect	66
5.4.6	Effect of heat treatment at 90°C	69
5.4.7	Kinetics of starch hydrolysis	70

5.4.8	Effect of inhibitor ( $\text{CuCl}_2$ ) on the amylase activity	73
5.4.9	Effect of amylase concentration	73
5.4.10	Complement experiments that can be performed	77
5.4.11	Notes	77
	References	78
<b>6</b>	<b>Determination of the pK of a Dye</b>	<b>79</b>
6.1	Definition of pK	79
6.2	Spectrophotometric Determination of pK	79
6.3	Determination of the pK of 4-Methyl-2-Nitrophenol	81
6.3.1	Experimental procedure	81
6.3.2	Solution	83
	References	87
<b>7</b>	<b>Fluorescence Spectroscopy Principles</b>	<b>88</b>
7.1	Jablonski Diagram or Diagram of Electronic Transitions	88
7.2	Fluorescence Spectral Properties	91
7.2.1	General features	91
7.2.2	Stokes shift	93
7.2.3	Relationship between the emission spectrum and excitation wavelength	94
7.2.4	Inner filter effect	95
7.2.5	Fluorescence excitation spectrum	95
7.2.6	Mirror-image rule	95
7.2.7	Fluorescence lifetime	96
7.2.8	Fluorescence quantum yield	101
7.2.9	Fluorescence and light diffusion	102
7.3	Fluorophore Structures and Properties	102
7.3.1	Aromatic amino acids	104
7.3.2	Cofactors	108
7.3.3	Extrinsic fluorophores	108
7.4	Polarity and Viscosity Effect on Quantum Yield and Emission Maximum Position	111
	References	113
<b>8</b>	<b>Effect of the Structure and the Environment of a Fluorophore on Its Absorption and Fluorescence Spectra</b>	<b>115</b>
	Experiments	115
	Questions	117
	Answers	119
	Reference	123
<b>9</b>	<b>Fluorophore Characterization and Importance in Biology</b>	<b>124</b>
9.1	Experiment 1. Quantitative Determination of Tryptophan in Proteins in 6 M Guanidine	124
9.1.1	Introduction	124



9.1.2	Principle	124
9.1.3	Experiment	125
9.1.4	Results obtained with cytochrome b <sub>2</sub> core	126
9.2	Experiment 2. Effect of the Inner Filter Effect on Fluorescence Data	127
9.2.1	Objective of the experiment	127
9.2.2	Experiment	127
9.2.3	Results	128
9.3	Experiment 3. Theoretical Spectral Resolution of Two Emitting Fluorophores Within a Mixture	130
9.3.1	Objective of the experiment	130
9.3.2	Results	132
9.4	Experiment 4. Determination of Melting Temperature of Triglycerides in Skimmed Milk Using Vitamin A Fluorescence	134
9.4.1	Introduction	134
9.4.2	Experiment to conduct	136
9.4.3	Results	136
	References	138
<b>10</b>	<b>Fluorescence Quenching</b>	<b>139</b>
10.1	Introduction	139
10.2	Collisional Quenching: the Stern–Volmer Relation	140
10.3	Different Types of Dynamic Quenching	145
10.4	Static Quenching	147
10.4.1	Theory	147
10.5	Thermal Intensity Quenching	154
	References	159
<b>11</b>	<b>Fluorescence Polarization</b>	<b>160</b>
11.1	Definition	160
11.2	Fluorescence Depolarization	162
11.2.1	Principles and applications	162
11.3	Fluorescence Anisotropy Decay Time	165
11.4	Depolarization and Energy Transfer	166
	References	167
<b>12</b>	<b>Interaction Between Ethidium Bromide and DNA</b>	<b>168</b>
12.1	Objective of the Experiment	168
12.2	DNA Extraction from Calf Thymus or Herring Sperm	168
12.2.1	Destruction of cellular structure	168
12.2.2	DNA extraction	168
12.2.3	DNA purification	169
12.2.4	Absorption spectrum of DNA	169
12.3	Ethidium Bromide Titration with Herring DNA	169
12.4	Results Obtained with Herring DNA	170
12.4.1	Absorption and emission spectra	170
12.4.2	Analysis and interpretation of the results	173

12.5	Polarization Measurements	177
12.6	Results Obtained with Calf Thymus DNA	179
12.7	Temperature Effect on Fluorescence of the Ethidium Bromide–DNA Complex	180
	References	182
<b>13</b>	<b><i>Lens culinaris</i> Agglutinin: Dynamics and Binding Studies</b>	<b>184</b>
13.1	Experiment 1. Studies on the Accessibility of I <sup>−</sup> to a Fluorophore: Quenching of Fluorescein Fluorescence with KI	184
13.1.1	Objective of the experiment	184
13.1.2	Experiment	184
13.1.3	Results	185
13.2	Experiment 2. Measurement of Rotational Correlation Time of Fluorescein Bound to LCA with Polarization Studies	187
13.2.1	Objective of the work	187
13.2.2	Polarization studies as a function of temperature	187
13.2.3	Polarization studies as a function of sucrose at 20°C	187
13.2.4	Results	189
13.3	Experiment 3. Role of $\alpha$ -L-fucose in the Stability of Lectin–Glycoproteins Complexes	190
13.3.1	Introduction	190
13.3.2	Binding studies	191
13.3.3	Results	192
	References	196
<b>14</b>	<b>Förster Energy Transfer</b>	<b>197</b>
14.1	Principles and Applications	197
14.2	Energy-transfer Parameters	202
14.3	Bioluminescence Resonance Energy Transfer	204
	References	208
<b>15</b>	<b>Binding of TNS on Bovine Serum Albumin at pH 3 and pH 7</b>	<b>210</b>
15.1	Objectives	210
15.2	Experiments	210
15.2.1	Fluorescence emission spectra of TNS–BSA at pH 3 and 7	210
15.2.2	Titration of BSA with TNS at pH 3 and 7	210
15.2.3	Measurement of energy transfer efficiency from Trp residues to TNS	211
15.2.4	Interaction between free Trp in solution and TNS	211
15.3	Results	211
<b>16</b>	<b>Comet Test for Environmental Genotoxicity Evaluation: A Fluorescence Microscopy Application</b>	<b>220</b>
16.1	Principle of the Comet Test	220
16.2	DNA Structure	220
16.3	DNA Reparation	221

16.4	Polycyclic Aromatic Hydrocarbons	222
16.5	Reactive Oxygen Species	223
16.6	Causes of DNA Damage and Biological Consequences	224
16.7	Types of DNA Lesions	225
16.7.1	Induction of abasic sites, AP, apurinic, or apyrimidinic	225
16.7.2	Base modification	225
16.7.3	DNA adducts	225
16.7.4	Simple and double-stranded breaks	225
16.8	Principle of Fluorescence Microscopy	225
16.9	Comet Test	227
16.9.1	Experimental protocol	227
16.9.2	Nature of damage revealed with the Comet test	227
16.9.3	Advantages and limits of the method	227
16.9.4	Result expression	231
	References	231
<b>17</b>	<b>Questions and Exercises</b>	<b>232</b>
17.1	Questions	232
17.1.1	Questions with shorts answers	232
17.1.2	Find the error	232
17.1.3	Explain	233
17.1.4	Exercises	234
17.2	Solutions	241
17.2.1	Questions with short answers	241
17.2.2	Find the error	243
17.2.3	Explain	243
17.2.4	Exercises solutions	244
	Index	253

**Color plate appears between pages 168 and 169**