

Contents

1	INTRODUCTION TO PRACTICAL TRAINING	7
1.1	Laboratory rules	7
1.2	Laboratory work safety	8
1.3	First aid in case of accident.....	9
2	BASICS OF WORKING IN A CHEMICAL LABORATORY	10
2.1	Biological sample processing	10
2.2	Principle and accuracy of pipetting.....	11
3	METHODS OF CHROMATOGRAPHY.....	15
3.1	Thin-layer chromatography	16
3.1.1	Separation of purine and pyrimidine bases by thin layer chromatography	18
3.2	Gel chromatography.....	20
3.2.1	Gel chromatography of haemolysed blood.....	21
4	PHOTOMETRIC ANALYSIS	23
4.1	Determination of salicylates	26
5	PROTEINS	29
5.1	Electrophoresis of serum proteins on agarose gel.....	29
5.2	Determination of total proteins by biuret reaction	31
5.3	Dialysis of blood	32
5.4	Osmosis.....	33
6	ENZYMES I	35
6.1	Enzymatic character of peroxidase reaction	35
6.2	Substrate specificity of α -amylase and sucrase.....	37
6.3	Inhibition of catalase activity by thiocyanate	39
7	ENZYMES II.....	41
7.1	Determination of Michaelis constant of model enzyme	41
7.1.1	Determination of Michaelis constant of alkaline phosphatase	43

8 ENZYMES III.....	46
8.1 Determination of lactate dehydrogenase activity in serum	51
8.2 Determination of alanine aminotransferase activity in serum.....	52
8.3 Determination of alkaline phosphatase activity in serum.....	54
8.4 Determination of creatine kinase activity in serum.....	55
9 SACCHARIDES.....	58
9.1 Chemical reaction of saccharides	58
9.1.1 Reducing properties of saccharides	58
9.1.2 Reactions of saccharides with strong acids.....	59
9.1.3 Reactions of polysaccharides.....	61
9.2 Determination of fasting glucose in serum and urine and after performing an oral glucose tolerance test (oGTT)	62
9.3 Determination of glucose in blood by a personal glucometer.....	66
9.4 Detection of glucose and ketone bodies in urine with diagnostic strips	67
10 LIPIDS.....	68
10.1 Chemical reaction of lipids.....	68
10.2 Determination of total cholesterol, HDL- and LDL-cholesterol and triacylglycerols in blood serum	70
10.2.1 Determination of total cholesterol in blood serum.....	72
10.2.2 Determination of HDL-cholesterol in blood serum	73
10.2.3 Determination of triacylglycerols in blood serum.....	75
10.2.4 Determination of LDL-cholesterol in blood serum	77
10.2.5 Electrophoresis of serum lipoproteins on agarose gel.....	79
11 AMINO ACIDS	82
11.1 Separation of amino acids by thin-layer chromatography.....	82
11.2 Determination of urea in serum and urine.....	83
12 BIOCHEMICAL PROCESSES IN GASTROINTESTINAL TRACT.....	86
12.1 Analysis of gastric juice	86
12.1.1 Qualitative examination of gastric juice.....	86

12.1.2	Quantitative examination of gastric juice	87
12.2	Influence of pH on pepsin activity.....	90
12.3	Determination of optimum temperature for trypsin.....	92
13	VITAMINS	95
13.1	Separation of a mixture of B vitamins by gel chromatography	95
14	XENOBIOTICS	98
14.1	Determination of acetylsalicylic acid.....	98
14.2	Determination of paracetamol.....	100
15	ACTIVE COMPOUNDS IN ORAL HYGIENE	102
15.1	Determination of sanguinarine and chelerythrine in dental care products	102
15.2	Determination of fluoride in dental care products using the ion selective electrodes	104
16	BIOCHEMISTRY OF THE ORAL CAVITY	107
16.1	Inorganic compounds in saliva	107
16.1.1	Qualitative determination of inorganic compounds in saliva	107
16.1.2	Demineralisation of hard tissues in the oral cavity	109
16.1.3	Determination of phosphorus in saliva	112
16.1.4	Determination of calcium in saliva.....	114
16.1.5	Determination of magnesium in saliva.....	116
16.2	Organic substances in saliva	118
16.2.1	Qualitative determination of organic substances in saliva.....	118
16.2.2	Electrophoresis of salivary proteins on agarose gel.....	121
16.2.3	Determination of enzymes in saliva	123
16.2.3.1	Determination of α -amylase in saliva	124
16.2.3.2	Determination of lactate dehydrogenase activity in saliva	126
16.2.3.3	Determination of aspartate aminotransferase activity in saliva	127
16.2.3.4	Determination of alkaline phosphatase activity in saliva	128
16.3	Buffering capacity of saliva	130
17	OXIDATIVE STRESS AND ANTIOXIDANT CAPACITY	132
17.1	Determination of total antioxidant activity of saliva	132

17.2	Determination of uric acid in saliva	134
17.3	Determination of vitamin C (ascorbic acid) in vitamin supplements and fruit juices.....	136
20.3	Determination of alkaline phosphatase activity in serum.....	211
20.4	Determination of creatine kinase activity in serum.....	213
26. SACCHARIDES		215
26.1	Chemical reaction of saccharides.....	215
26.1.1	Reducing properties of saccharides	215
26.1.2	Reactions of saccharides with strong acids	219
26.1.3	Conformations of polyaccharides.....	219
26.2	Determination of blood glucose concentration by performing oral glucose tolerance test (oGTT).....	221
26.3	Determination of glucose in blood by a manual glucometer, rebotracto.....	226
26.4	Detection of glucose and ketone bodies in urine by test strips.....	231
27. LIPIDS	235	
27.1	Determination of triglycerides in blood serum.....	235
27.2	Chemical reaction of lipoproteins similar to non-enzymatic reactions.....	236
27.3	Determination of triglycerides in blood serum.....	236
27.4	Determination of total cholesterol in blood serum.....	237
27.4.1	Determination of total cholesterol in blood serum.....	237
27.4.2	Determination of HDL-cholesterol in blood serum.....	238
27.4.3	Determination of triacylglycerols in blood serum in consecutive stages.....	238
27.4.4	Determination of LDL-cholesterol stage in sequential stages.....	239
27.4.5	Electrophoresis of serum lipoproteins in serum to determine.....	239
27.5	Analysis of triglycerides in serum to determine.....	240
27.6.1	Determination of amino acids in blood serum by the ninhydrin method	241
27.6.2	Determination of amino acids in blood serum by the bicinchoninic acid method	241
27.7	Determination of urea in blood serum by the diethyldithiocarbamate method	242
28.1	PHYSICAL PROCESSES IN GASTROINTESTINAL TRACT	246
28.1.1	Analyse of KINETIC, THERMODYNAMIC AND THERMOKINETIC PROCESSES.....	246
28.1.2.1	Qualitative examination of saliva mucus layer to mucus layer.....	246