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| Nota di contenuto | Principles and Applications of Fluorescence Spectroscopy; Contents; 1 Absorption Spectroscopy Theory; 1.1 Introduction; 1.2 Characteristics of an Absorption Spectrum; 1.3 Beer-Lambert-Bouguer Law; 1.4 Effect of the Environment on Absorption Spectra; References; 2 Determination of the Calcofluor White Molar Extinction Coefficient Value in the Absence and Presence of a1-Acid Glycoprotein; 2.1 Introduction; 2.2 Biological Material Used; 2.2.1 Calcofluor White; 2.2.2 a1-Acid glycoprotein; 2.3 Experiments; 2.3.1 Absorption spectrum of Calcofluor free in PBS buffer<br>2.3.2 Determination of e. value of Calcofluor White free in PBS buffer<br>2.3.3 Determination of Calcofluor White e. value in the presence of a1-acid glycoprotein; 2.4 Solution; References; 3 Determination of Kinetic Parameters of Lactate Dehydrogenase; 3.1 Objective of the Experiment; 3.2 Absorption Spectrum of NADH; 3.3 Absorption Spectrum of LDH; 3.4 Enzymatic Activity of LDH; 3.5 Kinetic Parameters; 3.6 Data and Results; 3.6.1 Determination of enzyme activity; 3.6.2 Determination of kinetic parameters; 3.7 Introduction to Kinetics and the Michaelis- |

Menten Equation; 3.7.1 Definitions
3.7.2 Reaction rates References; 4 Hydrolysis of p-Nitrophenyl-B-D-Galactoside with B-Galactosidase from E. coli; 4.1 Introduction; 4.2 Solutions to be Prepared; 4.3 First-day Experiments; 4.3.1 Absorption spectrum of PNP; 4.3.2 Absorption of PNP as a function of pH; 4.3.3 Internal calibration of PNP; 4.3.4 Determination of B-galactosidase optimal pH; 4.3.5 Determination of B-galactosidase optimal temperature; 4.4 Second-day Experiments; 4.4.1 Kinetics of p-nitrophenyl-B-D-galactoside hydrolysis with B-galactosidase; 4.4.2 Determination of the B-galactosidase concentration in the test tube 4.5 Third-day Experiments 4.5.1 Determination of Km and Vmax of B-galactosidase; 4.5.2 Inhibiton of hydrolysis kinetics of p-nitrophenyl-B-D-galactoside; 4.6 Fourth-day Experiments; 4.6.1 Effect of guanidine chloride concentration on B-galactosidase activity; 4.6.2 OD variation with guanidine chloride; 4.6.3 Mathematical derivation of Keq; 4.6.4 Definition of the standard Gibbs free energy, G.; 4.6.5 Relation between G. and G; 4.6.6 Relation between G. and Keq; 4.6.7 Effect of guanidine chloride on hydrolysis kinetics of p-nitrophenyl-B-D-galactoside; References
5 Starch Hydrolysis by Amylase 5.1 Objectives; 5.2 Introduction; 5.3 Materials; 5.4 Procedures and Experiments; 5.4.1 Preparation of a 20 g lS1starch solution; 5.4.2 Calibration curve for starch concentration; 5.4.3 Calibration curve for sugar concentration; 5.4.4 Effect of pH; 5.4.5 Temperature effect; 5.4.6 Effect of heat treatment at 90.C; 5.4.7 Kinetics of starch hydrolysis; 5.4.8 Effect of inhibitor (CuCl2) on the amylase activity; 5.4.9 Effect of amylase concentration; 5.4.10 Complement experiments that can be performed; 5.4.11 Notes; References; 6 Determination of the pK of a Dye
6.1 Definition of pK

Sommario/riassunto

Fluorescence spectroscopy is an important investigational tool in many areas of analytical science, due to its extremely high sensitivity and selectivity. With many uses across a broad range of chemical, biochemical and medical research, it has become an essential investigational technique allowing detailed, real-time observation of the structure and dynamics of intact biological systems with extremely high resolution. It is particularly heavily used in the pharmaceutical industry where it has almost completely replaced radiochemical labelling. Principles and Applications of Fluorescence