

Index

- accelerated flow techniques, 136
N-acetyl-L-phenylalanine-*p*-nitrophenyl ester, substrate for chymotrypsin, 165
 activated complex, 17
 activation energy, 16
 activator, 178, 189
 active site of enzymes, 25
 titration of chymotrypsin, 164; elastase, 164; factor Xa, 164; thrombin, 164; thymidylate synthetase, 164; trypsin, 164
 active site titration, 164
 active site competitive binding model, 208, 218
 active-site-directed effectors, 208
 acylation, 36
 acyl enzyme, 36
 of chymotrypsin, 36, 146
 of trypsin, 36, 146
 Adair model, 192
 adenyl cyclase, 169
 adrenalin, 169
 allosteric site, 174
 amplitude scaling of analogue computers, 266
 analogue computer programming, 255
 amplitude and time scaling, 266
 first order differential equations, 259
 host-parasite problem, 248, 263
 Michaelis-Menten equation, 262
 non-linear differential equations, 262
 relaxation experiment, 265
 second order differential equation, 261
 analogue computer units, 256-8, 307
 integrator, 256, 258, 309
 inverter, 256, 257, 307
 multiplier, 256, 258, 311
 potentiometer, 256, 257
 summer, 256, 257, 308
 analysis of coupled enzyme systems, 228
 lag phase, 231
 McClaurin polynomials, 235
 pre-steady-state, 239
 steady-state, 228
 analysis of kinetic results, 4
 differential method, 5
 integration method, 6
 statistical analysis, 285
 analysis of relaxation data, 163
 arginase inhibition, 57, 76
 Arrhenius equation, 16
 Arrhenius plots of α - and β -trypsin data, 101
 aspartate transcarbamylase, 171
 conformational changes, 180
 effectors, 177
 enzyme structure, 179
 kinetic behaviour, 178
 subunits, 179
 aspartokinase, feedback inhibition, 171
 ATP, effect on aspartate transcarbamylase, 171, 178

 Bi Bi mechanisms, 108, 118
 Ordered, 118: Haldane relationships, 121; initial-velocity equation, 119, 121; product inhibition studies, 121
 Ping Pong, 127: Haldane relationships, 130; initial-velocity equation, 129, 131; product inhibition studies, 131
 bimolecular reactions, 2
 binding curves, 174
 examples of, 178, 187, 188
 binding equations, 182
 heterotropic model, 189
 homotropic model, 183
 Monod, Wyman & Changeux model, 183
 normalised equations, 186
 binding sites, 174
 co-operative, 174
 equivalent, 184
 Bi Uni mechanism (Ordered), 115
 apparent maximum velocity, 118
 Haldane relationship, 116

- Bi Uni mechanism—continued**
 initial-velocity equation, 116
 reciprocal plots, 117
 Boltzmann expression, 16
- cascade process, 169
 chemical potential, 98
 chorismic acid-mutase prephenate dehydrogenase, 25
 chymotrypsin
 acylation step, 36
 deacylation step, 36
 hydrolysis of *p*-nitrophenyl acetate, 36
 inhibition by products, 77
 mechanism, 36
 titration with *p*-nitrophenyl-*N*²-acetyl-*N*¹-benzyl carbazate, 165
 circadian rhythms, 247, 253
 Cleland's analysis of multi-substrate reactions, 107
 co-factors, 26
 collision number, 17
 collision theory of reactions, 16
 comparison of enzyme parameters, 42
 specificity constant, 43
 competitive inhibition, 49
 hyperbolic, 53, 56
 integrated rate equation, 73
 linear, 49, 51
 parabolic, 51
 reciprocal plots, 51, 52, 53
 complex
 enzyme-substrate, 27
 Michaelis-Menten, 27
 transition-state, 19
 computation of enzyme parameters, 285
 computer program for determining K_m and V , 299
 curve fitting procedures, 285
 errors, 286
 least squares methods, 287: non-linear, 295; unweighted linear, 289; weighted linear, 292
 weighting factors, 293
 computer simulation, 251, 253, 254
 analogue, 253, 255
 digital, 269
 methods, 255, 269
 model building, 254
 of biochemical systems, 251
 of glycolytic pathway, 252
 concerted feedback inhibition, 173
 conformational changes in aspartate transcarbamylase, 180
 consecutive reactions, 11, 12
 conservation equations, 11, 28
 continuous flow methods, 136
 control enzymes, 168
 co-operativity
 negative, 176
 positive, 174
 coupled enzyme systems, 228
 analysis using McClaurin polynomials, 235
 example, 229
 steady-state analysis: two enzymes, 229; *n* enzymes, 233
 t^2 plot for two-enzyme system, 238
see also analysis of coupled enzyme systems
 covalent enzyme intermediates, 36, 165
 Crypto Ping Pong Uni Bi mechanism, 128
 CTP, effect on aspartate transcarbamylase, 171, 177
- deacylation, 36, 146
 degrees of freedom
 statistical analysis, 289
 transition state, 99
 denaturation of enzymes, 104
 enthalpy of, 104
 entropy of, 104
 pH effect, 83
 temperature effect, 104
 determinants, solution of linear simultaneous equations, 70
 deviation, 289
 mean, 289
 root mean square, 289
 sample standard, 289
 standard, 289
 sums of squares of, 289
 differential equations, 2
 first order, 13, 259
 non-linear, 262
 second order, 147, 261
 use of Laplace-Carson procedure, 279
 differential method, for analysis of kinetic results, 5
 digital computer simulation, 269
 flux equations, 270
 numerical integration, *see* numerical integration methods
 Dixon plots, 57
 competitive inhibition, 57
 non-competitive inhibition, 62
 double-intermediate mechanism, 35
 pre-steady-state: high enzyme

- concentration, 155; high substrate concentration, 146
 steady-state analysis, 35
- Eadie–Hofstee plots, 32, 33
 effector molecules, 171, 174, 177
 energy of activation, 16, 19, 99
 enthalpy of activation, 21, 99
 entropy of activation, 21, 99
 enzymes
 active sites, 25
 acylation of, 36
 binding to, 25
 catalytic effect of, 24
 co-factors of, 26
 complex with substrate, 27
 double-intermediate mechanism, 35
 effect of pH on, 83
 effect of temperature on, 97
 inhibition of, 48
 maximum velocity, 29
 Michaelis constant, 27
 Michaelis–Menten complex, 27
 Michaelis–Menten equation, 27
 pre-steady-state kinetics, 146
 primary structure, 25
 secondary structure, 25
 single-intermediate mechanism, 26
 steady-state theory, 28
 stereospecificity, 26
 tertiary structure, 25
 three-step mechanism, 35
 two-step mechanism, 26
 enzyme inhibition, 48
 competitive, 49: integrated rate equation, 73
 feedback, 48, 170
 hyperbolic competitive, 53
 irreversible, 49
 mixed, 65
 non-competitive, 58: integrated rate equation, 75
 parabolic competitive, 51
 partially non-competitive, 62
 by product, 69: integrated rate equation, 77
 reversible, 49
 uncompetitive, 63: integrated rate equation, 75
 enzyme titrants, 164
 epinephrine, 169
 equilibrium constant, 25
 equilibrium reactions, 11
 errors, effects on computation of enzyme parameters, 286, 296, 299
 Euler numerical integration method, 272
- fast reaction techniques, 135
 accelerated flow, 136
 continuous flow, 136
 flash-induced reactions, 144
 pressure jump, 143
 relaxation methods, 140
 stopped flow, 136
 temperature jump, 140
 feedback control, 48, 170
 feedback inhibition, 48, 170
 concerted, 173
 sequential, 173
 first order differential equations, 13, 259
 first order reactions, 2
 flash-induced reactions, 144
 5-Fluoro-2'-deoxyuridylate, 167
 flux equations, 270
 forward reaction rate, 11
 fractional activity, 57
 free energy changes in enzyme reactions, 24, 97
 free energy of activation, 21, 99
 frequency factor, 16
- Gaussian distribution, 288
 Gibbs–Helmholtz equation, 15
 glutamate dehydrogenase, 180
 effectors, 180, 182
 enzyme structure, 181
 kinetic behaviour, 182
 polymerisation, 180, 182
 subunits, 181
 glycolytic pathway, 252
 oscillations of, 251
- Haldane equations, 72
 Ordered Bi Bi, 121
 Ordered Bi Uni, 116
 Ordered Uni Bi, 113
 Ping Pong Bi Bi, 130
 Theorell–Chance, 124
 half life, 7
 Hanes plots, 31, 33
 heterotropic allosteric behaviour, 176
 Hill plots, 218
 Hofstee, *see* Eadie–Hofstee
 homoserine dehydrogenase, 172, 173
 homotropic allosteric behaviour, 176
 host–parasite model, 247
 solution using an analogue computer, 248, 263

322

hydrogen ion
 as competitive inhibitor, 86
 as uncompetitive inhibitor, 89
 hyperbolic competitive inhibition, 53, 56
 hysteretic enzymes, 220
 lag phase, 223

induced fit model of Koshland, 192, 199

inhibition, 48
 competitive, 49
 feedback, 48
 hyperbolic competitive, 53, 56
 integrated rate equations, 73, 75, 77
 irreversible, 49
 mixed, 65
 non-competitive, 58
 parabolic competitive, 51
 partially non-competitive, 62, 63
 product, 69
 uncompetitive, 63

initial velocity, 5, 28

initial-velocity equations
 Michaelis–Menten, 27
 Ordered Bi Bi, 120, 121
 Ordered Bi Uni, 115, 116
 Ordered Uni Bi, 110
 Ping Pong Bi Bi, 129
 Theorell–Chance, 123, 124

integrated rate equations
 competitive inhibition, 73
 Michaelis–Menten, 34
 non-competitive inhibition, 75
 product inhibition, 77
 uncompetitive inhibition, 75

integrating factor, 13

integration method, analysis of kinetic data, 6

integrator, analogue computer unit, 256, 258, 309

intrinsic binding constants, 184
 statistical weighting factors, 184, 209, 292

invertase, 26

inverter, analogue computer unit, 256, 257, 307

ionisation
 of enzyme, 85
 of enzyme–substrate complex, 88
 of enzyme and enzyme–substrate complex, 91
 of substrate, 94

isoenzymic control, 171

K_i , determination of inhibition constants, 57

Index

K_m
 definition, 29
 determination, 33, 34
 variation with pH, 86, 89, 92, 95

K_s , definition, 27

kinetic constants, 29
 comparison of values, 42

kinetic results, analysis of, *see* analysis of kinetic results

kinetics
 effects of added nucleophiles, 43
 effects of inhibitors, 48
 of multi-substrate reactions, 107
 pre-steady-state, 146
 steady-state, 28

King–Altman procedure, 37
 closed loops, 40
 computer analysis, 42
 patterns, 38, 40

lag phase, 223, 224

Laplace–Carson integral transforms, 279
 tables, 283
 theory, 279
 use, 151, 155, 281

least squares analysis, 287
 non-linear, 295
 unweighted linear, 289
 weighted linear, 292

linear regression analysis, 9, 289, 292

Lineweaver–Burk plots, 31, 33
 competitive inhibition, 51
 hyperbolic competitive inhibition, 53
 Michaelis–Menten equation, 31, 33
 mixed inhibition, 68
 non-competitive inhibition, 61
 Ordered Bi Bi mechanism, 117, 121
 Ordered Bi Uni mechanism, 117
 parabolic competitive inhibition, 53
 partially non-competitive inhibition, 64
 Ping Pong Bi Bi mechanism, 130
 Theorell–Chance mechanism, 124
 uncompetitive inhibition, 65
 weighting factors, 33, 292

Lotka equations, 247

maximum velocity, V , 29
 definition, 29
 determination, 31, 33

McClaurin polynomials, 235
 use in analysis of coupled enzyme systems, 235

- mean value, 288
 mechanisms, enzyme, 23
 multi-substrate, 107
 single-substrate, 26, 35
 metabolic control, 168, 169
N-methyl-*N*-toluene-*p*-sulphonyl-L-lysine β -naphthyl ester, titrant for trypsin, 165
 4-methylumbelliferyl-*p*-guanidinobenzoate, titrant for trypsin, 165
 Michaelis constant, 27
 definition, 27, 29
 determination, 31, 33
 Michaelis–Menten complex, 27
 Michaelis–Menten equation, 27
 solution using an analogue computer, 262
 mixed inhibition, 65
 steady-state equation, 66
 reciprocal plot, 68
 mixing chamber, 137
 molecular conversion, 173
 molecularity, 1
 bimolecular reactions, 2
 unimolecular reactions, 2
 Monod, Wyman & Changeux model, 183
 monomer–dimer system, 202
 effect on kinetics, 203
 multiple active sites, 183, 192, 213
 multiplier, analogue computer unit, 256, 258
 multi-step numerical integration methods, 275
 multi-substrate enzyme systems, 107
 Cleland's nomenclature, 107
 isomerisation, 108
 Ordered mechanisms, 108, 109, 115, 118
 Ping Pong mechanisms, 127
 product inhibition, 109, 114, 121, 131
 Random mechanisms, 125
 reactancy, 107
 sequential mechanisms, 108
 Theorell–Chance mechanism, 122
 negative co-operativity, 176
p-nitrocinnamoyl- α -chymotrypsin, *cis*–*trans* stereoisomerism, 145
p-nitrophenyl - N^2 - acetyl - N^1 - benzyl carbazate titration of chymotrypsin, 165
 non-competitive inhibition, 58
 integrated rate equation, 75
 partially non-competitive, 62
 reciprocal plots, 61, 64
 non-linear differential equations, 255
 solution using analogue computer, 262
 non-linear least squares analysis, 295
 normal distribution, 288
 normal equations, 291
 normalised binding equations, 186, 191
 nucleophiles, effect on steady-state kinetics, 43
 numerical integration methods, 270
 one-step, 272: Euler, 272; Runge–Kutta, 274
 multi-step, 275: predictor–corrector, 275
 one-step methods for numerical integration, *see under* numerical integration methods
 operational molarity, 164
 operator, Laplace–Carson, *see* Laplace–Carson integral transforms
 Ordered reactions, 108
 Bi Bi, 118
 Bi Uni, 115
 Ter Ter, 132
 Uni Bi, 109
 order of reactions, 2
 first order, 2
 pseudo first order, 4
 second order, 2
 zero order, 3
 oscillatory behaviour, 246
 cross-coupling of reactions, 250
 glycolytic pathway, 252
 Lotka equations, 247
 parabolic competitive inhibition, 51
 Dixon plot, 58
 reciprocal plot, 52
 parameters of enzymes
 comparison of, *see* comparison of enzyme parameters
 computation of, *see* computation of enzyme parameters
 partially non-competitive inhibition, 62
 reciprocal plot, 64
 periodic forcing functions, 143
 pH, effect on rate of enzyme reactions, 83
 irreversible effects, 83
 optimum, 86, 90
 reversible effects, 83
 variation of K_m , 86, 89, 92, 95
 variation of V , 88, 92

- Ping Pong mechanisms, 108
 Bi Bi, 127: Haldane relationship, 130;
 initial-velocity equation, 129; product
 inhibition studies, 131
 Bi Bi Uni Uni, 132
 Bi Uni Uni Bi, 132
 Crypto Uni Bi, 128
 Hexa Uni, 132
 pK_a , of ionising groups in active site of
 trypsin, 85
 Planck's constant, 20
 polymerisation, 180, 182, 200
 effect on binding curves, 203
 monomer-dimer, 202, 206
 monomer-hexamer, 203
 positive co-operativity, 174
 potentiometer, analogue computer unit,
 256, 257
 predictor-corrector numerical integra-
 tion method, 275
 pressure jump, 143
 pre-steady-state kinetics, analysis, 146
 double-intermediate mechanism, 146
 high enzyme concentration, 155
 high substrate concentration, 146
 pre-steady-state kinetics, methods, 136
 flash-induced reactions, 144
 rapid flow techniques, 136: accelerated
 flow, 136; continuous flow, 136;
 stopped flow, 136
 relaxation methods, 140: pressure
 jump, 143; temperature jump, 140
 prey-predator model, 247, 263
 primary structure of enzymes, 25
 probability, 288, 290
 probability factor, 17
 product inhibition, 69
 integrated rate equation for single sub-
 strate reaction, 77
 of multi-substrate systems, 109, 114,
 121, 131
 programming, analogue computer, *see*
 analogue computer programming
 pseudo first order reaction, 4
- R_s , deviation from rectangular hyper-
 bola, 174
 Random mechanisms, 108, 125
 rapid random, 127
 rapid flow techniques, 136
 accelerated flow, 136
 continuous flow, 136
 stopped flow, 136
 rapid reactions, 135
 flash-induced reactions, 144
 rapid flow techniques, 136
 relaxation methods, 140
 rate constant, 2
 definition, 2
 dimensions, 4
 transition state theory, 21
 rate equation, 2
 competitive inhibition, 50
 Michaelis-Menten equation, 27, 34
 mixed inhibition, 66
 non-competitive inhibition, 59
 product inhibition, 71
 uncompetitive inhibition, 64
 rate-limiting step, 36
 reactivity, 108
 reaction,
 equilibrium, 11
 sequential, 11, 12
 reaction order, *see* order of reactions
 reaction pathway, free energy changes,
 24, 97
 reaction rates, 1
 effect of pH on enzyme reactions, 83
 effect of temperature, 14, 97
 forward, 15
 reverse, 15
 reciprocal plots, 31, 33
 competitive inhibition, 51
 effect of added nucleophiles, 46
 hyperbolic competitive inhibition, 53
 Michaelis-Menten equation, 31
 mixed inhibition, 68
 non-competitive inhibition, 61
 parabolic competitive inhibition, 52
 partially non-competitive inhibition,
 64
 two-substrate reactions, 117, 130:
 Ordered Bi Bi, 115, 121; Ordered Bi
 Uni, 115; Ping Pong Bi Bi, 130;
 Theorell-Chance, 124
 uncompetitive inhibition, 65
 weighting factors, 33, 293
 rectangular hyperbola, 30
 regression line, 9, 33, 290
 regulatory enzymes, 168
 activator, 178
 active site competitive binding model,
 208
 aspartate transcarbamylase, 177
 binding equations, 183
 feedback inhibition, 170
 glutamate dehydrogenase, 180
 heterotropic effects, 176

- homotropic effects, 176
 hysteretic effects, 220
 isoenzymic control, 171
 kinetic behaviour, 174, 212
 Koshland's induced fit model, 192
 Monod, Wyman & Changeux model, 183
 negative co-operativity, 176
 polymerisation model, 200
 positive co-operativity, 174
 relaxation, 136
 kinetics, 157
 techniques, 140: analysis of data, 163;
 periodic forcing function, 143; pres-
 sure jump, 143; step forcing func-
 tion, 142; temperature jump, 140
 time, 158: of $A \rightleftharpoons B$ process, 158; of
 $A + B \rightleftharpoons C$ process, 160; of $A \rightleftharpoons$
 $B \rightleftharpoons C$ process, 161
 relaxation experiment, analogue com-
 puter solution, 265
 reversible effects
 due to inhibitor, 48
 due to pH changes, 83
 due to temperature changes, 97, 104,
 105
 reversibility of reactions, product inhibi-
 tion, 69
 rhythms, circadian, 247, 253
 root mean square deviation, 289
 Runge–Kutta method of numerical inte-
 gration, 274

 sample standard deviation, 289
 scaling of analogue computer problems,
 266
 amplitude, 266
 time, 267
 secondary structure of enzymes, 25
 second order differential equations, 147,
 261
 second order reaction, 2
 sequential feedback inhibition, 173
 sequential mechanism, multi-substrate
 reactions, 108
 sequential reactions, 11, 12
 serine proteinases, 36
 important amino acids, 84
 mechanism, 36
 sigmoidal binding curves, 174
 simulation, *see* computer simulation
 single-intermediate mechanism, 26
 specificity constant, 43
 standard deviation, 289

 standard free energy change, 21, 25, 98
 statistical analysis, 287
 linear least squares, 289
 non-linear least squares, 295
 weighted linear least squares, 292
 statistical factors,
 intrinsic binding constants, 184
 weighting factors for reciprocal plots,
 33, 293
 steady-state kinetics, 28
 coupled enzyme systems, 228
 effect of added nucleophiles, 43
 effect of inhibitors, 48
 of multi-substrate reactions, 107
 steady-state theory, 28
 step function in relaxation experiments,
 140, 142
 stereospecificity of enzyme reactions, 26
 steric factor, collision theory, 17
 stiff differential equations, 275, 276
 stopped flow method, 136
 substrate, 24, 27
 effect of ionisation on the rate of reac-
 tion, 94
 complex with the enzyme, 27
 subunits,
 of aspartate transcarbamylase, 179
 of glutamate dehydrogenase, 181
 summer, analogue computer unit, 256,
 257
 sums of squares of deviation, 289

 t^2 plot of data for a two-enzyme coupled
 system, 238
 Taylor series expansion, 296
 non-linear least squares analysis,
 295
 temperature,
 effect on enzyme-catalysed reactions,
 97
 effect on equilibrium constant, 15
 effect on reaction rates, 14
 temperature jump method, 140, 158
 tertiary structure, 25
 Theorell–Chance mechanism, 122
 Haldane relationship, 124
 initial-velocity equation, 123
 product inhibition studies, 124
 thermal denaturation of enzymes, 104
 enthalpy change, 104
 entropy change, 104
 thiol subtilisin, 226
 three-step mechanism, 35

326

Index

- three-step mechanism—*continued*
 pre-steady-state kinetics, 146: high enzyme concentration, 155; high substrate concentration, 146
 steady-state kinetics, 36
 threonine deaminase, 177
 thymidylate synthetase, 167
 time scaling of analogue computers, 266, 267
 titration of enzyme solutions, 164
N-toluene-*p*-sulphonyl-L-lysine β -naphthylester, substrate for trypsin, 165
 transformed variables, weighting factors, 293
 transient phase of coupled reactions, 234, 239
 transient state kinetics, 135
 transient time, 234
 transition state analogue, 24, 49
 transition state complex, 24, 49, 97
 degrees of freedom, 99
 effect of catalyst, 20
 enthalpy of activation, 21, 99
 entropy of activation, 21, 99
 entropy loss on formation, 100
 free energy of activation, 21, 99
 transition state theory, 18
 rate constant, 21
 trypsin, 36, 101, 146, 164
 Arrhenius plots, 101
 catalysed hydrolysis of esters, 36
 determination of operational molarity, 164
 pK_a of ionising groups in active site, 85
- two-step mechanism, 26
 uncompetitive inhibition, 63
 integrated rate equation, 75
 reciprocal plot, 65
 steady-state equation, 64
 Uni Bi mechanism, 109
 definition of maximum velocity, 111
 definition of Michaelis constant, 111
 definition of inhibition constant(s), 112
 equilibrium constant, 113
 Haldane equation, 113
 product inhibition studies, 114
 unimolecular reactions, 2
 unweighted linear least squares analysis, 289
- van't Hoff isochore, 15
 variance, 289
 variation of
 K_m with pH, 86, 89, 92, 95
 V with pH, 88, 92
 V with temperature, 101
 velocity
 apparent maximum, 116
 initial, 5, 28
 maximum, 29
- Woolf plot, 32, 33
- zero order reactions, 4, 26