## CONTENT

1. Principles of enzyme assay and kinetic studies  
   1. Introduction  
   2. Behaviour of assays  
   3. The effects of enzyme concentration  
   4. Expression of enzyme activity  
   5. The effects of substrate concentration  
   6. Experimental approaches  

2. Photometric assays  
   1. Introduction  
   2. Absorption  
   3. Turbidimetry  
   4. Fluorescence  

3. Radiometric assays  
   1. Introduction  
   2. Techniques  
   3. Experimental design  
   4. Problems and pitfalls  
   5. Automation of assays  
   6. The advantages of radiometric methods of enzyme assay  

Acknowledgements  
References  

4. High performance liquid chromatographic assays  
   1. Introduction  
   2. Theory of HPLC  
   3. Retention mechanism  
   4. Instrumentation  
   5. Detectors  
   6. Practical considerations  
   7. Application of HPLC to enzymatic analysis  

References  

5. Electrochemical assays: polarography  
   1. Introduction  
   2. Polarographic principles  
   3. Polarographic techniques  
   4. Polarographic enzyme assays  

References
6. Electrochemical assays: the oxygen electrode
   1. Introduction 181
   2. Theory and principles 181
   3. Current/voltage relationships 182
   4. Sensitivity 182
   5. Calibration 183
   6. Electrode systems 185
   7. Polarographic assays 186
   References 190

7. Electrochemical assays: the pH-stat
   1. Introduction 191
   2. Principles and theoretical basis of pH-stat methodology 192
   3. Commercial and custom-made pH-stat assemblies: automation, computer control, and special applications 194
   4. General pH-stat procedure and chemical principles and experimental protocols for some individual enzymes 199
   5. A systematic error in pH-stat assays of enzymes in haemolysates 213
   6. Concluding comment 214
      Acknowledgements 214
      References 214

8. Detection of enzymatic activity after polyacrylamide gel electrophoresis and agarose gel isoelectric focusing
   1. Introduction 217
   2. Preparation of sample and material 218
   3. Preparation of slab gels for zymography 220
   4. Gel formulations 223
   5. Electrophoresis 227
   6. Staining 228
   7. Troubleshooting the electrophoresis 229
   8. Preparation of electrophoretically-separated enzymes for detection 230
   9. Blotting/elution/renaturation 230
   10. Detection of enzymes in gels 235
   11. Practical examples for enzyme detection 237
   12. Other enzymology-after-electrophoresis techniques 250
      Acknowledgement 251
      References 251

9. Techniques for enzyme extraction
   1. Introduction: scope of chapter 255
   2. Disruption of tissues and cells 256
   3. Protection of enzyme activity 262
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Assays of enzymes in unfractionated cell-extracts</td>
<td>268</td>
</tr>
<tr>
<td>5. Assays of enzymes in unfractionated cell-extracts</td>
<td>268</td>
</tr>
<tr>
<td>6. In situ assays using permeabilization techniques</td>
<td>271</td>
</tr>
<tr>
<td>7. Concluding remarks</td>
<td>273</td>
</tr>
<tr>
<td>References</td>
<td>273</td>
</tr>
<tr>
<td>10. Statistical analysis of enzyme kinetic data</td>
<td>277</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>277</td>
</tr>
<tr>
<td>2. Preliminary considerations</td>
<td>278</td>
</tr>
<tr>
<td>3. General strategy for determination of Km and Vmax</td>
<td>281</td>
</tr>
<tr>
<td>4. Calculation of Km and Vmax using graphs</td>
<td>286</td>
</tr>
<tr>
<td>5. Calculation of Km and Vmax using computerized least-squares-fit methods</td>
<td>291</td>
</tr>
<tr>
<td>6. Multi-substrate reactions</td>
<td>297</td>
</tr>
<tr>
<td>7. Inhibition and the determination of the inhibitor constant (Ki values)</td>
<td>302</td>
</tr>
<tr>
<td>8. Uses for the standard deviations</td>
<td>305</td>
</tr>
<tr>
<td>9. The analysis of Hill plots</td>
<td>312</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>313</td>
</tr>
<tr>
<td>References</td>
<td>313</td>
</tr>
<tr>
<td>11. Buffers and the determination of protein concentrations</td>
<td>317</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>317</td>
</tr>
<tr>
<td>2. Buffers and pH</td>
<td>317</td>
</tr>
<tr>
<td>3. Methods for protein determination</td>
<td>326</td>
</tr>
<tr>
<td>References</td>
<td>334</td>
</tr>
<tr>
<td>Appendix Suppliers of specialist items</td>
<td>337</td>
</tr>
<tr>
<td>Enzyme index</td>
<td>343</td>
</tr>
<tr>
<td>Subject index</td>
<td>347</td>
</tr>
</tbody>
</table>