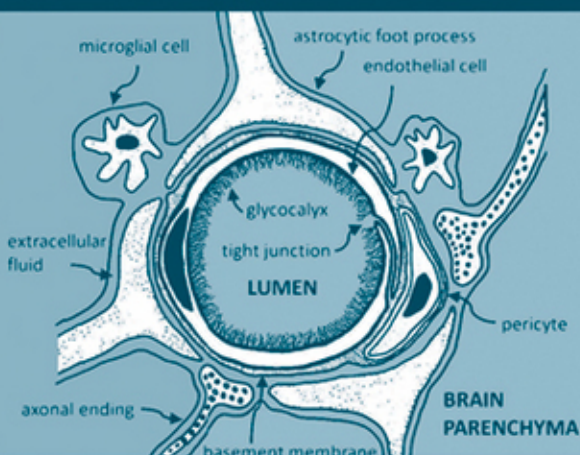


Second Edition

Absorption and Drug Development

*Solubility, Permeability,
and Charge State*

Alex Avdeef



ABSORPTION AND DRUG DEVELOPMENT

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Solubility, Permeability, and Charge State

Second Edition

ALEX AVDEEF

in-ADME Research

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CONTENTS

Preface	xxiii
Preface to the First Edition	xxvii
List of Abbreviations	xxxii
Nomenclature	xxxv
Commercial Trademarks	xli
1 Introduction	1
1.1 Bulldozer Searching for a Needle in the Haystack?, 1	
1.2 As the Paradigm Turns, 4	
1.3 Screen for the Target or ADME First?, 5	
1.4 ADME and Multimechanism Screens, 6	
1.5 ADME and the Medicinal Chemist, 7	
1.6 The “Absorption” in ADME, 8	
1.7 It Is Not Just a Number, It Is a Multimechanism, 9	
References, 9	
2 Transport Model	12
2.1 Permeability–Solubility–Charge State and pH-Partition Hypothesis, 12	
2.2 Properties of the Gastrointestinal Tract (GIT), 17	
2.3 pH Microclimate, 22	
2.4 Intracellular pH Environment, 23	

- 2.5 Tight Junction Complex, 23
- 2.6 Structure of Octanol, 23
- 2.7 Biopharmaceutics Classification System, 25
- References, 26

3 pK_a Determination 31

- 3.1 Charge State and the pK_a , 32
- 3.2 Methods of Choice for the Determination of the pK_a , 34
- 3.3 Titration with a Glass-Membrane pH Electrode, 34
- 3.4 Equilibrium Equations and the Ionization Constant, 38
- 3.5 “Pure Solvent” Activity Scale, 41
- 3.6 Ionic Strength and Debye–Hückel/Davies Equation, 41
- 3.7 “Constant Ionic Medium” Activity Scale, 43
 - 3.7.1 Constant Ionic Medium Activity Scale, f_X^γ , 44
 - 3.7.2 Example of the Constant Ionic Medium Activity Scale Treatment, 44
- 3.8 Temperature Dependence of pK_a Values, 47
 - 3.8.1 Thermodynamics of Temperature Dependence of pK_a , 48
 - 3.8.2 Predicting pK_a at 37°C from Measured Values at 25°C, 52
- 3.9 Electrode Calibration and Standardization, 55
 - 3.9.1 Overview: From Millivolts to pH to p_cH , 56
 - 3.9.2 One-Buffer Operational Calibration of Electrode and Automatic Temperature Compensation, 56
 - 3.9.3 Four-Parameter Procedure for Standardizing pH Electrode: α , k_S , j_H , j_{OH} Parameters, 60
 - 3.9.3.1 Blank Titration, 61
 - 3.9.3.2 Buffer-Capacity Enhanced Standardization Titrations, 63
 - 3.9.4 Blank Titrations Assess the State of the Measurement System, 63
 - 3.9.5 Frequency of Electrode Standardizations, 64
 - 3.9.6 Effects of Ionic Strength on α , k_S , j_H , j_{OH} , 64
 - 3.9.7 Effects of Temperature on α , k_S , j_H , j_{OH} , 64
- 3.10 Bjerrum Plot: Most Useful Graphical Tool in pK_a Analysis, 66
 - 3.10.1 Derivation of the Bjerrum Function, 66
 - 3.10.2 Diagnostic Uses of the Bjerrum Plots, 69
 - 3.10.2.1 Confirming the Number of pK_a and the Value of n_H , 69
 - 3.10.2.2 Correcting Residual Acidity/Basicity Error, 69
 - 3.10.2.3 Correcting Sample Concentration Error, 71
 - 3.10.2.4 Recognizing Electrode Calibration Errors, 71
 - 3.10.2.5 Errors in Titrant Concentration, 71
 - 3.10.2.6 Consequences of Errors in Ionic Strength, 72
 - 3.10.2.7 Evidence of Precipitation, 72

- 3.10.2.8 pK_a “Ghosts”, 72
- 3.10.2.9 Overlapping pK_a Values, 73
- 3.10.3 More Than One Substance: Subtracting Effects of Dissolved Carbon Dioxide, 73
- 3.10.4 Case Examples, 75
- 3.10.5 Bjerrum’s Lasting Contribution, 75
- 3.11 Cosolvent Methods for pK_a Determination of Practically Insoluble Substances, 78
 - 3.11.1 Traditional Cosolvent Extrapolation Procedure: $p_s K_a$ versus wt% Cosolvent, 79
 - 3.11.2 Yasuda–Shedlovsky Cosolvent Extrapolation Procedure: $p_s K_a + \log[H_2O]$ versus $1/\epsilon$, 83
 - 3.11.3 Which Method Is Better to Use: Traditional or Yasuda–Shedlovsky?, 86
 - 3.11.4 Precipitation in Mixed-Solvent Titration, 87
 - 3.11.5 Electrode Standardization in Mixed Solvents, 88
 - 3.11.6 Yasuda–Shedlovsky Ancillary Data, 91
 - 3.11.7 Recommended $p_s K_a$ Titration Protocol, 92
 - 3.11.8 Use of Cosolvents for Water-Soluble Molecules, 93
- 3.12 Other Methods for pK_a Measurement, 96
 - 3.12.1 Spectrophotometric Measurements, 96
 - 3.12.2 Capillary Electrophoresis (CE) Measurements, 99
 - 3.12.3 Chromatographic pK_a Measurement, 100
 - 3.12.4 pK_a Measurement by PAMPA (Parallel Artificial Membrane Permeability Assay), 100
 - 3.12.5 pK_a Determination from Shake-Flask $\log D_{OCT}$ versus pH Profiles, 101
 - 3.12.6 Comparison of Methods, 101
- 3.13 pK_a Microconstants, 102
- 3.14 pK_a Compilations, 107
- 3.15 pK_a Prediction Programs, 107
- 3.16 Database of pK_a (25°C and 37°C), 107
- Appendix 3.1 Quick Start: Determination of the pK_a of Codeine, 127
 - A3.1.1 Data Collection, 127
 - A3.1.2 Data Processing, 128
- Appendix 3.2 Tutorial for Measurements with Glass-Membrane pH Electrode, 130
 - A3.2.1 The pH Electrode Is an Electrochemical Sensor, 131
 - A3.2.2 Calibration of Glass-Membrane pH Electrodes Requires Standard Buffers, 132
 - A3.2.3 Sources of Error in the Most Accurate pH Determinations, 133
 - A3.2.4 Interlaboratory Comparison of pH Measurement Using Glass-Membrane pH Electrodes, 133
 - A3.2.5 Three pH Scales in Use, 134

- Appendix 3.3 pH Convention Adopted by IUPAC and Supported by NIST, 137
- Appendix 3.4 Liquid-Junction Potentials (LJP), 140
- A3.4.1 LJP Minimized with Equi-ionic Cell Design (Best Case, but Inconvenient), 142
 - A3.4.2 Constant but Small LJP with Constant Ionic Medium Cell (Practical Case), 143
 - A3.4.3 Largest LJP Errors with Zero Ionic Strength Media (Worst Case), 144
 - A3.4.4 Summary, 145
- Appendix 3.5 pK_a Refinement by Weighted Nonlinear Regression, 146
- A3.5.1 Function Minimized in the Weighted Nonlinear Regression Analysis, 146
 - A3.5.2 Overview of the Refinement Procedure, 146
 - A3.5.3 Weighting Scheme and Goodness-of-Fit (GOF), 147
 - A3.5.4 Mass Balance Equations and “Local” Refinement, 148
 - A3.5.4.1 Sample Calculation of the Initial Point Prior to Titrant Addition, 150
 - A3.5.4.2 Sample Calculation of the Titration Curve after Volume of Titrant Addition, 150
 - A3.5.4.3 Jacobian Method in “Local” Refinement, 152
 - A3.5.5 Normal Equations and “Global” Refinement, 154
 - A3.5.6 Automatic Ionic Strength Compensation, 155
 - A3.5.7 Reactant and Titrant Concentration Factor Refinement, 156
 - A3.5.8 Multisubstance Refinement, 157
 - A3.5.9 *In situ* Standardization of the pH Electrode, 157
- Appendix 3.6 Molality to Molarity Conversion, 157
- References, 158

4 Octanol–Water Partitioning

174

- 4.1 Overton–Hansch Model, 175
- 4.2 Tetrad of Equilibria, 175
- 4.3 Conditional Constants, 177
- 4.4 $\log P$ Data Sources, 178
- 4.5 $\log D$ Lipophilicity Profile, 178
- 4.6 Ion-Pair Partitioning, 183
 - 4.6.1 Partitioning of Quaternary Ammonium Drugs, 183
 - 4.6.2 Common-Ion Effect and $\log D$ of Multiprotic Drugs, 183
 - 4.6.3 Summary of Charged-Species Partitioning in Octanol–Water, 186
 - 4.6.4 Ion-Pair Absorption of Ionized Drugs: Fact or Fiction?, 187

- 4.7 Micro- $\log P$, 187
- 4.8 Methods for $\log P$ Determination, 188
 - 4.8.1 HPLC Methods, 188
 - 4.8.2 High-Throughput Methods, 188
 - 4.8.3 Other $\log P$ Methods, 188
- 4.9 Dyrssen Dual-Phase Titration $\log P$ Method, 189
 - 4.9.1 Brief History of the Dual-Phase Titration Method, 189
 - 4.9.2 Dual-Phase Method, 190
 - 4.9.3 Dual-Phase Bjerrum Plots, 190
 - 4.9.3.1 Monoprotic Molecules, 190
 - 4.9.3.2 Multiprotic Molecules, 191
 - 4.9.4 Validation, 194
- 4.10 Ionic Strength Dependence of $\log P$, 194
- 4.11 Temperature Dependence of $\log P$, 194
- 4.12 Calculated versus Measured $\log P$ of Research Compounds, 194
- 4.13 $\log D$ versus pH Case Study: Procaine Structural Analogs, 196
- 4.14 Database of Octanol–Water $\log P^N$, $\log P^I$, and $\log D_{7.4}$, 201
- References, 209

5 Liposome–Water Partitioning

220

- 5.1 Biomimetic Lipophilicity, 221
- 5.2 Tetrad of Equilibria and Surface Ion-Pairing (SIP), 221
- 5.3 Data Sources, 222
- 5.4 Location of Drugs Partitioned into Bilayers, 222
- 5.5 Thermodynamics of Partitioning: Entropy- or Enthalpy-Driven?, 223
- 5.6 Electrostatic and Hydrogen Bonding in a Low Dielectric Medium, 224
- 5.7 Water Wires, H^+/OH^- Currents, and Permeability of Amino Acids and Peptides, 227
- 5.8 Preparation Methods: MLV, SUV, FAT, LUV, ET, 228
- 5.9 Experimental Methods, 229
- 5.10 Prediction of $\log P_{MEM}$ from $\log P_{OCT}$, 229
- 5.11 $\log D_{MEM}$, $diff \log P_{MEM}$, and Prediction of $\log P_{MEM}^{SIP}$ from $\log P_{OCT}^I$, 233
- 5.12 Three Indices of Lipophilicity: Liposomes, IAM, and Octanol, 238
- 5.13 Getting It Wrong from One-Point $\log D_{MEM}$ Measurement, 239
- 5.14 Partitioning into Charged Liposomes, 240
- 5.15 pK_a^{MEM} Shifts in Charged Liposomes and Micelles, 240
- 5.16 Prediction of Absorption from Liposome Partition Studies?, 241
- 5.17 Database of $\log P_{MEM}$ and $\log P_{MEM}^{SIP}$, 242
- References, 245

6 Solubility**251**

- 6.1 It's Not Just a Number, 252
- 6.2 Why Is Solubility Measurement Difficult?, 252
 - 6.2.1 Poor Wettability, 253
 - 6.2.2 Formation of Aggregates and Micelles, 253
 - 6.2.3 Equilibration Time and Metastable Gel States, 254
 - 6.2.4 Polymorphism and Amorphous States, 254
- 6.3 Mathematical Models for Solubility–pH Profiles, 255
 - 6.3.1 Monoprotic Weak Acid, HA (or Base, B), 255
 - 6.3.2 Diprotic Ampholyte, XH_2^+ , 256
 - 6.3.3 Gibbs pK_a (“ pH^{max} ”) and the Tetrad of Equilibria, 257
 - 6.3.4 Aggregation Reactions in Solubility Measurement, 262
 - 6.3.4.1 Sample Derivation of Ionic Aggregation of a Weak Acid, 265
 - 6.3.4.2 Solubility Equations for Other Cases Involving Aggregation Reactions, 265
 - 6.3.5 Complexation Reactions in Solubility Measurement (Phase-Solubility Method), 265
 - 6.3.5.1 Simple 1:1 Complexation, 265
 - 6.3.5.2 Simple 2:1 Ligand–Drug Complexation, 267
 - 6.3.5.3 Simple 1:1 Complexation with Deprotonation of the Complex, 267
 - 6.3.5.4 Summary of Complexation Equations, 269
 - 6.3.6 Micellar Binding Reactions in Solubility Measurement, 269
- 6.4 Experimental Methods, 270
 - 6.4.1 Saturation Shake-Flask (“Gold Standard” SSF Method), 270
 - 6.4.2 Turbidimetric (DMSO-Containing “Kinetic” Ranking Methods), 270
 - 6.4.3 Micro-Dissolution for Solubility Measurement (DMSO-Free μ DISS Method), 271
 - 6.4.4 DMSO-Containing Thermodynamic 96- and 384-Well Plate Methods, 272
 - 6.4.4.1 HTS Methods, 272
 - 6.4.4.2 Self-Calibrating Microsolubility ($\leq 1\%$ v/v DMSO– μ SOL Method), 272
 - 6.4.5 DMSO-Free Thermodynamic 96- and 384-Well Plate Methods, 277
 - 6.4.5.1 Lyophilization (GeneVac), 277
 - 6.4.5.2 Partially Automated Solubility Screening (PASS), 277
 - 6.4.5.3 Miniaturized Shake-Flask (MSF), 277
 - 6.4.5.4 Dual-Phase Potentiometric Titration (DTT), 278

- 6.4.6 Facilitated Dissolution Method (FDM), 283
- 6.4.7 Can Solubility Depend on the Amount of Excess Solid?, 285
- 6.4.8 Excipient and Solubilizing Agent Screening, 285
- 6.4.9 Need for Accurate pK_a Determinations, 287
- 6.5 Correction for the DMSO Effect by the “ Δ -Shift” Method, 287
 - 6.5.1 DMSO Binding to the Uncharged Form of a Compound, 287
 - 6.5.2 Ionizable Compound Binding by Nonionizable Excipients, 288
 - 6.5.3 Results of Aqueous Solubility Determined from the Δ -Shifts, 289
- 6.6 Case Studies (Solubility–pH Profiles), 289
 - 6.6.1 Carboxylic Weak Acids, 289
 - 6.6.1.1 Diclofenac, 289
 - 6.6.1.2 Gemfibrozil, 291
 - 6.6.1.3 Ibuprofen, 291
 - 6.6.1.4 Ketoprofen, 291
 - 6.6.1.5 Mefenamic Acid, 292
 - 6.6.1.6 Naproxen, 292
 - 6.6.1.7 Prostaglandin $F_{2\alpha}$, 292
 - 6.6.2 Noncarboxylic Weak Acids, 292
 - 6.6.2.1 Glibenclamide, 292
 - 6.6.2.2 Phenytoin, 294
 - 6.6.3 Weak Bases, 294
 - 6.6.3.1 Dipyrindamole, 295
 - 6.6.3.2 Papaverine, 296
 - 6.6.3.3 Terfenadine, 296
 - 6.6.4 High-Throughput Excipient/Solubilizer Screening, 297
 - 6.6.4.1 Excipient/Solubilizer Concentrations, 297
 - 6.6.4.2 Excipient/Solubilizer Solubility Profiles, 297
 - 6.6.4.3 Drug Effects, 301
 - 6.6.4.4 Summary, 301
 - 6.6.5 Additional Case Studies of Excipient/Solubilizer Effects on Solubility of Sparingly Soluble Drugs, 302
 - 6.6.5.1 Ketoprofen with Sodium Lauryl Sulfate (SLS), 302
 - 6.6.5.2 Mefenamic Acid and Hydroxypropyl- β -Cyclodextrin (HP- β -CD), 302
 - 6.6.5.3 Mefenamic Acid and Sodium Taurocholate (NaTC), 304
 - 6.6.6 Salt Solubility: Amount of Excess Solid Can Determine Solubility, 306
 - 6.6.6.1 Chlordiazepoxide, 306
 - 6.6.6.2 Flurbiprofen, 306
 - 6.6.6.3 Terfenadine, 306

- 6.7 Limits of Detection—Precision versus Accuracy, 306
- 6.8 Data Sources and the “Ionizable-Drug Problem,” 308
- 6.9 Database of $\log S_0$, 308
- References, 310

7 Permeability—PAMPA 319

- 7.1 Permeability in the Gastrointestinal Tract, 320
- 7.2 Historical Developments in Permeability Models, 323
 - 7.2.1 Lipid Bilayer Concept, 323
 - 7.2.2 Black Lipid Membranes (BLM), 326
 - 7.2.3 Microfilter as Supports, 327
 - 7.2.4 Octanol-Impregnated Filters with Controlled Water Pores, 332
 - 7.2.5 Relationship Between Permeability and Partition Coefficients, 333
- 7.3 Rise of PAMPA—A Useful Tool in Early Drug Discovery, 336
 - 7.3.1 The Original Egg Lecithin Model: PAMPA-EGG, 336
 - 7.3.2 Dioleoylphosphatidylcholine Model: PAMPA-DOPC, 336
 - 7.3.3 Hexadecane Model: PAMPA-HDM, 338
 - 7.3.4 Biomimetic Model: PAMPA-BM, 339
 - 7.3.5 Hydrophilic Filter Membrane Model: PAMPA-HFM, 341
 - 7.3.6 Double-Sink PAMPA Model: PAMPA-DS, 342
- 7.4 PAMPA-HDM, -DOPC, -DS Models Compared, 343
 - 7.4.1 *In Combo* Approach for Relating PAMPA Models, Using Abraham Descriptors, 344
 - 7.4.2 Intrinsic Permeability of the Test Compounds by the pK_a^{FLUX} Method, 349
 - 7.4.3 DS > DOPC > HDM Permeability Ranking, 347
 - 7.4.4 Abraham Analysis of PAMPA-HDM, -DOPC, and -DS Models, 347
 - 7.4.5 *In Combo* Analysis of the Differences Between the Three PAMPA Models, 349
 - 7.4.6 Practical Considerations in Using HDM and DS Models, 351
- 7.5 Modeling Biological Membranes, 354
 - 7.5.1 Lipid Compositions in Biological Membranes, 354
 - 7.5.2 Permeability–pH Considerations, 356
 - 7.5.3 Membrane Retention (Iso-pH without Chemical Sink), 357
 - 7.5.4 Role of Serum Proteins, 359
 - 7.5.5 Effects of Cosolvents, Bile Acids, and Other Surfactants, 360
 - 7.5.6 Ideal Model, 361

- 7.6 Permeability–pH Relationship and the Mitigating Effect of the Aqueous Boundary Layer, 362
 - 7.6.1 Permeability–pH (Iso-pH DOPC Model), 362
 - 7.6.2 Permeability–pH (Gradient-pH Double-Sink Model), 365
 - 7.6.3 Stirring with Orbital Shakers, 365
 - 7.6.4 Individual-Well Stirring, 368
 - 7.6.5 Relationship Between Membrane and ABL Permeability, 368
 - 7.6.6 pH-Dependence of Permeability: Correcting PAMPA Permeability for the ABL and Charge Effects by the pK_a^{FLUX} Method, 370
 - 7.6.6.1 Membrane-Limiting Permeation (Hydrophilic Molecules), 374
 - 7.6.6.2 ABL-Limiting Permeation (Lipophilic Molecules): pK_a^{FLUX} Method, 374
 - 7.6.6.3 Analysis of Stirring Effects Using the pK_a^{FLUX} Method, 376
 - 7.6.7 ABL Hydrodynamic Model Commonly Used in Cellular Studies, 376
 - 7.6.8 Thickness of the ABL, 381
 - 7.6.9 Why Is the Empirical Hydrodynamic Model α -Factor Not Equal to 0.5?, 382
 - 7.6.10 Determining P_0 of Nonionizable Molecules or in Membrane-Limited Permeation, 384
 - 7.6.11 Determination of ABL Permeability from Permeability of Lipid-Free Microfilters, 385
 - 7.6.12 Estimation of h_{ABL} from pH Measurements Near the Surface of Membranes, 385
 - 7.6.13 Errors in the Determination of P_0 by the pK_a^{FLUX} Method, 386
- 7.7 pK_a^{FLUX} -Optimized Design (*pOD*), 386
- 7.8 Cosolvent PAMPA, 389
 - 7.8.1 pK_a^{FLUX} Method in Cosolvent Media, 390
 - 7.8.2 Acid–Base *In Combo* Permeability Model, 396
 - 7.8.3 Cosolvent Extrapolation Method, 397
- 7.9 UV versus LC/MS Detection, 397
 - 7.9.1 UV Data, 398
 - 7.9.2 LC/MS Data, 398
- 7.10 Assay Time Points, 400
- 7.11 Buffer Effects, 402
- 7.12 Apparent Filter Porosity, 404
 - 7.12.1 Apparent Porosity, 404
 - 7.12.2 Apparent Porosity in PAMPA-DS and PAMPA-HDM, 406
 - 7.12.3 Recalculation Using the Apparent Porosity, 407

- 7.13 PAMPA Errors: Intra-Plate and Inter-Plate Reproducibility, 407
- 7.14 Human Intestinal Absorption (HIA) and PAMPA, 409
 - 7.14.1 Max-PAMPA Binning Methods, 409
 - 7.14.2 Sum-PAMPA Binning Methods, 410
 - 7.14.3 *Human* Jejunal Permeability to Predict Human Intestinal Absorption, 412
 - 7.14.4 MAD-PAMPA Method Integrating Solubility and Permeability, 413
- 7.15 Permeation of Permanently Charged Molecules, 416
 - 7.15.1 Charged-Species Transport from Cellular and Liposomal Models, 417
 - 7.15.2 The PAMPA Evidence for the Permeation of Charged Drugs, 421
- 7.16 Permeation of Zwitterions/Ampholytes—*In Combo* PAMPA, 424
 - 7.16.1 An *In Combo* PAMPA Model Incorporating Paracellular Contribution, 426
 - 7.16.1.1 Paracellular Permeability Analysis (Pore Radius, Porosity–Pathlength, Electric Potential Gradient), 426
 - 7.16.1.2 Zwitterion/Neutral Intrinsic Permeability, 428
 - 7.16.1.3 Absorption Prediction with *In Combo* PAMPA, 430
 - 7.16.2 Absorption Curve as a Function of P_e^{INT} , 430
- 7.17 PAMPA in Formulation: Solubilizing Excipient Effects, 433
 - 7.17.1 *In Vivo* Absorption as a Function of pH, 436
 - 7.17.2 Nonlinear Weighted Regression Reanalysis of Rat Absorption–pH Curves, 437
 - 7.17.3 Solubility and Permeability in Flux, 439
 - 7.17.4 Absorption–Excipient–pH Classification Gradient Map, a Flux Function, 440
 - 7.17.5 The Conversion of Intrinsic Data to Intestinal pH 5.0, 6.2, and 7.4 Conditions, 440
 - 7.17.6 Absorption and the Underlying Permeability and Solubility, 442
 - 7.17.6.1 PAMPA–Excipient–pH CGM, 442
 - 7.17.6.2 Solubility–Excipient–pH CGM, 442
 - 7.17.6.3 Solubility-Product “Salt Ceiling,” 442
 - 7.17.7 Absorption–Excipient–pH CGM, 443
 - 7.17.8 HP- β -CD Effects, 444
 - 7.17.9 pH-Partition Hypothesis “Inversion,” 446
 - 7.17.10 Comparisons with Drug–Cyclodextrin *In Vivo* Bioavailability Data, 448
- 7.18 Database of Double-Sink PAMPA $\log P_0$, $\log P_m^{6.5}$, and $\log P_m^{7.4}$, 448

- Appendix 7.1 Quick Start: Double-Sink PAMPA of Metoprolol, 460
 - A7.1.1 Optimizing Assay Protocol, 461
 - A7.1.2 PAMPA-DS (Gradient-pH) Assay, 461
 - A7.1.3 Data Processing, 463
- Appendix 7.2 Permeability Equations, 465
 - A7.2.1 Thin-Membrane Model (without Retention), 467
 - A7.2.2 Iso-pH Equations with Membrane Retention, 470
 - A7.2.2.1 Without Precipitate in Donor Wells and Sink Condition in Receiver Wells, 470
 - A7.2.2.2 Sink Condition in Receiver Wells, 474
 - A7.2.2.3 Precipitated Sample in the Donor Wells, 475
 - A7.2.3 Gradient-pH Equations with Membrane Retention, 476
 - A7.2.3.1 Single-Sink: Eq. (A7.28), in Absence of Chemical Sink (Serum Protein or Surfactant in Receiver Wells), 478
 - A7.2.3.2 Double-Sink: Eq. (A7.28), in the Presence of Chemical Sink (Serum Protein or Surfactant in Receiver Wells), 479
 - A7.2.3.3 Simulation Examples, 480
 - A7.2.3.4 Gradient-pH Summary, 481
- Appendix 7.3 PAMPA Paramembrane Water Channels, 481
 - A7.3.1 PAMPA Permeability Equation Taking into Account Paramembrane Contribution, 482
 - A7.3.2 PAMPA Determinations, 482
 - A7.3.3 Water Pores in PAMPA Membrane Barrier, 484
- References, 484

8 Permeability: Caco-2/MDCK

499

- 8.1 Permeability in the Gastrointestinal Tract, 500
 - 8.1.1 Human Jejunal Permeability, 500
 - 8.1.2 Environment of the GIT Site of Absorption, 501
 - 8.1.2.1 Intestinal Surface Accessibility and the “Smooth Tube” Approximation, 502
 - 8.1.2.2 Aqueous Boundary Layer (ABL), 502
 - 8.1.2.3 Paracellular Aqueous Channels, 503
 - 8.1.2.4 pH Gradients, 504
 - 8.1.2.5 Normalizing *In Vitro*–*In Vivo* Conditions, 504
 - 8.1.3 Carrier-Mediated Transporters and PAMPA, 504
- 8.2 Cell-Based *In Vitro* Permeability Model, 505
 - 8.2.1 Limitations of a Single-pH Measurement and Ways to Overcome Them, 506
 - 8.2.1.1 ABL-Limited Transport May Not Correlate with *In Vivo* Permeability, 506

- 8.2.1.2 Paracellular Transport May Be Important for Low-Permeable Molecules, 509
- 8.2.1.3 How to Extract P_C from P_{app} in a Single-pH Permeability Measurement, 509
- 8.2.1.4 How to Extract P_C from P_{app} from Multiple-pH Permeability Measurement, 510
- 8.2.2 Characteristic Paracellular Parameters in a Standardized Cell Culture, 510
 - 8.2.2.1 Model Equation to Determine Cell-Based *In Vitro* Paracellular Parameters, 510
 - 8.2.2.2 Refinement of the *In Vitro* Paracellular Permeability Parameters, 513
- 8.3 *In Situ* Human Jejunum Permeability (HJP) Model, 514
 - 8.3.1 Model Equation to Determine HJP Paracellular Parameters, 514
 - 8.3.2 Refinement of the HJP Parameters, 515
- 8.4 Passive Intrinsic Permeability Coefficients of Caco-2 and MDCK Compared, 515
- 8.5 Theory (Stage 1): Paracellular Leakiness and Size Exclusion in Caco-2, MDCK, and 2/4/A1 Cell Lines, 516
 - 8.5.1 Selection of Paracellular Markers, 517
 - 8.5.2 Paracellular Model Nonlinear Regression Analysis, 517
 - 8.5.3 Iso-Paracellular Profiles, 519
 - 8.5.4 Ranking by Size Exclusion, 522
 - 8.5.5 Confirming the Filter Porosity Experimentally, 522
 - 8.5.5.1 Filter Permeability, P_f , in Caco-2 Assays, 522
 - 8.5.5.2 Determination of Filter Porosity, 523
- 8.6 Theory (Stage 2): Regression Method for *In Vitro* Cellular Permeability, 524
 - 8.6.1 Two Stages of Analysis and the Dynamic Range Window (DRW), 524
 - 8.6.2 Refinement of the *In Vitro* Cellular Permeability Parameters, 525
- 8.7 Case Studies of Cell-Based Permeability as a Function of pH, 525
 - 8.7.1 *In Vitro* Permeability Measurements Are Insufficiently Standardized, 525
 - 8.7.2 Pretreatment of *In Vitro* Permeability Measurements to Improve IVIVC, 526
 - 8.7.2.1 Treatment of the Two-pH Caco-2 Data to Determine “True” Cell Permeability, 526
 - 8.7.2.2 Treatment of the Multiple-pH Caco-2 Data to Determine “True” Cell Permeability, 528
 - 8.7.2.2.1 Alfentanil, 528
 - 8.7.2.2.2 Cimetidine, 529
 - 8.7.2.2.3 Verapamil, 529

- 8.7.2.2.4 Atenolol, Metoprolol, and
Propranolol, 530
- 8.7.2.2.5 Indomethacin and Retinoic Acid, 532
- 8.8 Human Jejunal Permeability Predicted Directly from Caco-2/
MDCK, 533
 - 8.8.1 Effective Human Jejunal Permeability Data Sources, 533
 - 8.8.2 Biophysical Model Regression Analysis of Human Jejunal
Permeability, 534
 - 8.8.3 Refined Effective Surface Area Expansion Factor, 534
 - 8.8.4 Transport Analysis for Drugs in the Human
Jejunum, 541
 - 8.8.4.1 Paracellular Route, 542
 - 8.8.4.2 ABL-Limited Transcellular Route, 544
 - 8.8.4.3 Transcellular Route, 545
 - 8.8.5 Polyethylene Glycol Permeability, 545
 - 8.8.6 The “Leakiness” of the Human Jejunum Compared with
That of *In Vitro* Models, 548
 - 8.8.7 Applications of the Biophysical Model, 548
 - 8.8.8 Biophysical Model Summary, 550
- 8.9 Caco-2/MDCK Database and Its *In Combo* PAMPA
Prediction, 550
 - 8.9.1 Caco-2/MDCK Database, 551
 - 8.9.2 *In Combo* Model Building Approach, 561
 - 8.9.3 Caco-2/MDCK Passive Permeability Prediction
Model, 561
- References, 563

9 Permeability: Blood–Brain Barrier

575

- 9.1 The Blood–Brain Barrier: A Key Element for Drug Access to the
Central Nervous System, 576
- 9.2 The Blood–Brain Barrier, 576
 - 9.2.1 The BBB Environment, 577
 - 9.2.2 Lipid Composition of the BBB, 578
 - 9.2.3 Transporters at the BBB, 578
- 9.3 Noncellular BBB Models, 580
 - 9.3.1 $\log P_{\text{OCT}}$, 580
 - 9.3.2 $\Delta \log P$, 581
 - 9.3.3 Air–Water Partition Coefficients and Molecular
Cross-Sectional Area, 582
 - 9.3.4 Black Lipid Membrane (BLM) Models, 583
 - 9.3.5 PAMPA-BBB, 583
 - 9.3.6 Selectivity Coefficients (SC) and the Solubility-Diffusion
Theory, 584
- 9.4 *In Vitro* BBB Cell-Based Models, 586

- 9.5 *In Vivo* BBB Models, 589
 - 9.5.1 Brain/Plasma Ratio: K_p (also Known as B/P and log BB)—Extent of Penetration, 589
 - 9.5.2 Mouse Brain Uptake Assay (MBUA), 589
 - 9.5.3 Rodent *In Situ* Brain Perfusion: Rate of Transport, 590
 - 9.5.4 Microdialysis, 592
- 9.6 Paradigm Shift, 592
 - 9.6.1 Extent of Brain Penetration (Concentration in the Brain Extracellular Fluid ECF), 592
 - 9.6.2 Equilibration Half-Time in Physiologically Based Pharmacokinetics (PBPK), 603
 - 9.6.3 Unbound Fractions in Brain and Plasma, 603
 - 9.6.4 Brain Penetration Classification (BPC), 604
- 9.7 *In Silico* BBB Models, 608
- 9.8 Biophysical Analysis of *In Vitro* Endothelial Cell Models, 608
 - 9.8.1 Computational Method, 608
 - 9.8.2 Selection of *In Vitro* BMEC Permeability Data from Various Laboratories, 609
 - 9.8.3 Extracting Aqueous Hydrodynamic Contributions from Measured Permeability, 610
 - 9.8.4 *In Vitro* BMEC Transendothelial and Intrinsic Permeability, P_c and P_0 , 610
 - 9.8.5 Dynamic Range Window (DRW), 610
 - 9.8.6 Results of Paracellular and Aqueous Boundary Layer Permeability Analysis, 612
 - 9.8.6.1 Transporter Effects, 614
 - 9.8.6.2 Refined Paracellular Parameters, 614
 - 9.8.6.3 Ranking by Leakiness, 615
 - 9.8.6.4 Size Exclusion, 616
 - 9.8.7 Transendothelial Cell Membrane Permeability Analysis, 616
 - 9.8.7.1 IVIVC Analysis with Uncorrected (P_e) Data, 616
 - 9.8.7.2 Improved IVIVC Analysis Using P_0 Data, 618
- 9.9 *In Situ* Brain Perfusion Analysis of Flow, 618
 - 9.9.1 Flow-Limit Window (FLW), Where PS Cannot Be Determined from K_{in} , 618
 - 9.9.2 Approaches to Overcome the Possible Limitation of the Crone–Renkin Equation, 619
 - 9.9.3 Is There an ABL at the BBB?, 620
 - 9.9.4 The pH-CRE (Crone–Renkin Equation) Flow Correction Method, 621
 - 9.9.5 Paracellular Permeability at the BBB, 623
 - 9.9.6 Capillary versus Planar Hydrodynamic Effects in Permeability Assays, 623
 - 9.9.7 Effective BBB Permeability versus Lipophilicity, 625

9.9.8	<i>In Situ</i> Rat Brain Perfusion Measurement as a Function of pH, 627	
9.9.9	Determined Mean Cerebrovascular Flow Velocity: Literature Comparisons, 631	
9.9.10	pH-CRE Method Recommendations: Maprotiline at pH 7.4 and 6.5, 631	
9.10	<i>In Combo</i> PAMPA–BBB Model for Passive BBB Permeability, 631	
9.10.1	<i>In Silico</i> Model Building Software and the <i>In Combo</i> Strategy, 632	
9.10.2	Linear Free Energy Relations (LFER) Descriptors, 658	
9.10.3	PAMPA–BBB Selectivity Coefficients by Charge Classes, 659	
9.10.4	Abraham LFER and <i>In Combo</i> PAMPA Models, 659	
9.10.5	“External” Set Comparisons, 662	
	References, 663	
10	Summary and Some Simple Approximations	681
	Index	685

PREFACE

In the nine years since the first edition of *Absorption and Drug Development*, a number of advances have been made, especially in the permeability methods. Several PAMPA models based on targeted lipid formulations have been described by pharmaceutical researchers. New data processing procedures were introduced to interpret permeability–pH dependence (gradient- and iso-pH) in PAMPA, as well as in cultured epithelial cell lines (e.g., Caco-2, MDCK), in primary endothelial cultured cells [e.g., porcine brain microcapillary endothelial cells (BMEC) and human BMEC], and in the rodent *in situ* brain perfusion model. The first PAMPA models specifically directed at modeling the blood–brain barrier (BBB) permeability have been reported. PAMPA models for skin penetration have been described. Even areas of solubility data analysis have seen some progress.

In the first edition, the pK_a and solubility sections were sketchy, more like reviews than book chapters. The original permeability chapter was long and focused on the early stages of the evolution of what came to be known as the Double-Sink PAMPA method. Upon reflection, the need for a more balanced coverage was evident.

In this second edition, most of the original PAMPA material has been scrapped and has been replaced by descriptions and applications of models based on the more recent research described in literature, drawing on over 30 PAMPA-related papers published from the group at *p*ION INC that the author headed. Also, two new chapters have been added: Chapter 8 (Permeability: Caco-2/MDCK) and Chapter 9 (Permeability: Blood–Brain Barrier). The pK_a chapter has been vastly expanded. The potentiometric technique is covered comprehensively, but the treatment is still slim on UV and other

methods. The new origin-shifted Yasuda–Shedlovsky (OSYS) method revealed some novel insights about how to treat insoluble acids and bases differently. The solubility chapter has been brought up to date with many examples of the treatment of practically insoluble test compounds. It was tempting to add a new chapter on dissolution, but the size of the book would have exceeded the planned limit. It was thought that a separate treatment of solubility–dissolution would best be left to a future project.

All of the database tables have been reviewed and updated with more values. The pK_a table now has more than 900 entries, with many determined at 37°C. New tables have been added to each of the permeability chapters, with extensive listings of Double-Sink PAMPA, PAMPA–BBB, Caco-2/MDCK, multispecies BMEC, and *in situ* brain perfusion (PS) values. The introductory chapter, Chapter 1, has been updated, since the R&D paradigm of pharmaceutical research has undergone important changes since the first edition.

Based on the content of the first edition, the author twice taught an informal 10-week course at King's College, London. There were other, smaller, teaching presentations at the University of Helsinki on two separate occasions. The notion of the book serving an educational purpose was recurrent. Several pharmacy and pharmaceutical sciences university departments have courses in physical pharmacy and pharmaceutics based on Martin's classical textbook, *Physical Pharmacy and Pharmaceutical Sciences* (now in its sixth edition). This is an excellent and comprehensive text for a two-semester introductory graduate course. The author taught selected topics from it as a guest lecturer at Northeastern University, Boston, on a couple of occasions. However, one cannot learn how to do physicochemical measurements (e.g., pK_a , solubility, and permeability) from Martin's book alone. Therefore, a more advanced treatment of the physicochemical methods related to drug absorption is needed for pharmaceutics graduate students, especially those headed for careers in the pharmaceutical industry. The author has received comments from several professors who have used parts of the first edition of *Absorption and Drug Development* to supplement advanced pharmaceutics courses. Slanting the second edition toward an educational textbook was very tempting, but due to time constraints it was decided to leave that for a future separate add-on booklet to accompany the main text. Preparing useful questions and answers is not a minor project. The second edition still can be used to augment advanced graduate courses in pharmaceutics and as a reference for researchers in pharmaceutical R&D (and in some instances in agrochemical, environmental, and related industries). The author welcomes more feedback from academics and other readers about how the book can be improved, both as a teaching guide and as a reference.

The second edition is organized into 10 chapters. Chapter 1 describes the physicochemical measurement needs of pharmaceutical R&D, in a quickly

changing environment. Chapter 2 defines the flux model, based on Fick's laws of diffusion, in terms of solubility, permeability, and charge state (pK_a), and lays the foundation for the rest of the book. Chapter 3 covers the topic of ionization constants: how to measure pK_a constants well and quickly, and which strategies to use. It has been completely rewritten from the short previous version. Chapter 4 is about experimental methods of measuring partition coefficients, $\log P$ and $\log D$. It contains a description of the Dyrssen dual-phase potentiometric method, which remains the "gold standard" technique for measuring $\log P$ of ionizable molecules, having the unique 10-orders-of-magnitude range ($\log P$ from 2 to +8). Chapter 5 considers the special topic of partition coefficients where the lipid phase is made of liposomes formed from bilayers of phospholipids. This chapter remains largely the same. Chapter 6 covers solubility measurements and has been broadly expanded. Chapter 7 describes PAMPA, the high-throughput artificial membrane permeability method originally introduced by Manfred Kansy and co-workers from Hoffmann-La Roche. The chapter has been substantially revised and remains a deep account of the rapidly developed important topic. Many hundreds of original measurements are tabulated in the chapter. Chapter 8 considers permeability measurements using epithelial cell models, such as Caco-2 and MDCK. Chapter 9 does so with endothelial cultured cell models, and it attempts to correlate these to animal *in situ* brain perfusion measurements of luminal permeability. Chapter 10 concludes with simple physicochemical property approximations. More than 1350 references and well over 200 drawings and 200 pages of tables substantiate the book as an extensively documented reference work.

I have many colleagues to thank for their thoughts, criticism, guidance, and opportunities for collaborations: Joan Abbott, Mike Abraham, Per Artursson, David Begley, Stephanie Bendels, Christel Bergström, Marival Bermejo, Li Di, Jennifer Dressman, Beate Escher, Bernard Faller, Holger Fischer, Norman Ho, Pranas Japertas, Paulius Jurgutis, Manfred Kansy, Ed Kerns, Stefanie Krämer, Chris Lipinski, Sibylle Neuhoff, Alanas Petrauskas, Tom Raub, Jean-Michel Scherrmann, Abu Serajuddin, Kiyohiko Sugano, Krisztina Takács-Novák, Bernard Testa, Björn Wagner, Han van de Waterbeemd, and Shinji Yamashita. I owe gratitude to many others, including my former colleagues at *p*ION INC and Sirius Analytical Instruments Ltd. I left *p*ION at the beginning of the year, to start *in-ADME* Research (ADME software and consulting) and to finish writing this book.

Salvatore Cisternino, Markus Fridén, Margareta Hammarlund-Udanaes, Krisztina Takács-Novák, and Kin Tam were most kind to read various chapters as the book was being written and offered many helpful suggestions, for which I am especially grateful.

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I would also like to thank Joyce Saltalamachia for her love and support, as she put up with a lot during my 12 months of writing and other things.

ALEX AVDEEF

*Cambridge, Massachusetts
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PREFACE TO THE FIRST EDITION

This book is written for the practicing pharmaceutical scientist involved in ADME measurements, who needs to communicate with medicinal chemists persuasively, so that new synthesized molecules will be more “drug-like.” ADME is all about “a day in the life of a drug molecule” (absorption, distribution, metabolism, excretion). Specifically, this book attempts to describe the state of the art in measurement of ionization constants (pK_a), oil–water partition coefficients ($\log P/\log D$), solubility, and permeability (artificial phospholipid membrane barriers). Permeability is covered in considerable detail, based on a newly developed methodology known as PAMPA (Parallel Artificial Membrane Permeability Assay).

These physical parameters form the major components of physicochemical profiling (the “Absorption” in ADME) in the pharmaceutical industry, from drug discovery through drug development. However, there are opportunities to apply the methodologies in other fields, particularly the agrochemical and environmental industries. In addition, new applications to augment animal-based models in the cosmetics industry may be interesting to explore.

It has been the author’s observation that graduate programs in pharmaceutical sciences often neglect adequately to train students in these classical solution chemistry topics. Often young scientists in pharmaceutical companies are assigned the task of measuring some of these properties in their projects. Most find the learning curve somewhat steep. In addition, experienced scientists in mid careers come upon the topic of physicochemical profiling for the first time, and they find few resources to draw upon outside of the primary literature.

The idea for a book on the topic has morphed through various forms, beginning with focus on the subject of metal-binding to biological ligands, when the author was a postdoc in Professor Ken Raymond's group at University of California, Berkeley. When the author was an Assistant Professor of Chemistry at Syracuse University, every time the special topics course on speciation analysis was taught, more notes were added to the "book." After five years, more than 300 pages of hand-scribbled notes and derivations accumulated, but no book emerged. Some years later, a subsection of the original notes acquired a binding and saw light in the form of *Applications and Theory Guide to pH-Metric pK_a and $\log P$ Measurement*, out of the early effort in the start-up of Sirius Analytical Instruments Ltd., in Forest Row, a charming four-pub village at the edge of Ashdown Forest, south of London. At Sirius, the author was involved in teaching a comprehensive three-day training course to advanced users of pK_a and $\log P$ measurement equipment manufactured by Sirius. The trainees were from pharmaceutical and agrochemical companies, and they shared many new ideas during the courses. Over the last decade, Sirius has standardized the measurement of pK_a values in the pharmaceutical and agrochemical industries. Some 50 courses later, the practice continues at another young company, *pION*, located along high-tech highway 128, north of Boston, Massachusetts. The list of topics has expanded over the last 12 years, to cover solubility, dissolution, and permeability, as new instruments were developed. Last year, an opportunity to write a review article came up, and a bulky piece appeared in *Current Topics in Medicinal Chemistry*, entitled Physicochemical Profiling (Solubility, Permeability and Charge State). In reviewing that manuscript, Cynthia Berger (*pION*) said that with a little extra effort, "this could be a book." Further encouragement came from Bob Esposito of John Wiley & Sons. My colleagues at *pION* were kind about my taking a sabbatical in England, to focus on the writing. I was privileged to join Professor Joan Abbott's neuroscience laboratory at King's College London for three months, where I conducted an informal 10-week graduate short course on the topics of this book, as the material was freshly written. After hours, it was my pleasure to jog with my West London Hash House Harrier friends. As the chapter on permeability was being written, my very capable colleagues at *pION* were quickly measuring permeability of membrane models freshly inspired by the book writing. It is due to their efforts that Chapter 7 is loaded with so much original data, out of which emerged the "Double-Sink" PAMPA model for predicting human intestinal permeability. Per Nielsen (*pION*) reviewed the manuscript as it slowly emerged, with a keen eye. Many late-evening discussions with him led to freshly inspired insights, now imbedded in various parts of the book.

The book is organized into eight chapters. Chapter 1 describes the physicochemical needs of pharmaceutical research and development. Chapter 2 defines the flux model, based on Fick's laws of diffusion, in terms of solubility, permeability, and charge state (pK_a), and lays the foundation for the rest of the book. Chapter 3 covers the topic of ionization constants: how to measure