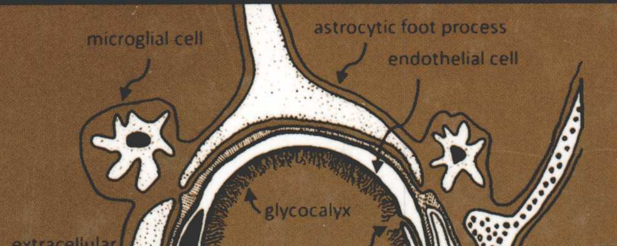
Second Edition

Absorption and Drug Development

Solubility, Permeability, and Charge State

Alex Avdeef



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ALEX AVDEEF
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ABSORPTION AND DRUG DEVELOPMENT

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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In this second edition, most of the original PAMPA material has been scrapped and has been replaced by descriptions and applications of models based on the more recent research described in literature, drawing on over 30 PAMPA-related papers published from the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.

Joan Abbott is a dear friend and has been a generous host on a number of occasions that I spent time writing and recharging in her group at King's College, London.

I am especially privileged and grateful to have known Manfred Kansy as a friend for the last 20 years.

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
September 2011

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, *p*ION, 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 (*p*ION) 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 *p*ION 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 *p*ION 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 (*p*ION) 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 pK_a values well and quickly, and which methods to use. Bjerrum analysis is revealed as the "secret" weapon behind the most effective approaches. 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 that truly is the "gold standard" method for measuring log *P* of ionizable molecules, having the unique 10 orders of magnitude range (log *P* from 2 to +8). High-throughput methods are also described. Chapter 5 considers the special topic of partition coefficients where the lipid phase is made of liposomes formed from vesicles made of bilayers of phospholipids. Chapter 6 dives into solubility measurements. A unique approach, based on the Dissolution Template Titration method, has demonstrated capabilities to measure solubilities as low as one nanogram per milliliter. In addition, high-throughput microtiter plate UV methods for determining "thermodynamic" solubility constants are described. At the ends of Chapters 3–6, an effort has been made to collect tables of critically selected values of the constants of drug molecules, the best available values. Chapter 7 describes PAMPA, the high-throughput method recently introduced by Manfred Kansy et al. of Hoffmann–La Roche. Chapter 7 is the first thorough account of the topic and takes up almost half of the book. Nearly 4000 original measurements are tabulated in the chapter. Chapter 8 concludes with simple rules. Over 600 references and well over 100 drawings substantiate the book.

Professor Norman Ho (University of Utah) was very kind to critically read the permeability chapter and comment on the various derivations and concepts. His unique expertise on the topic spans many decades. His thoughts and advice (15 pages of handwritten notes) inspired me to rewrite some of the sections in that chapter. I am very grateful to him. I am grateful to other colleagues at *p*ION who expertly performed many of the measurements of solubility and permeability, which are presented in the book: Chau Du, Jeffrey Ruell, Melissa Strafford, Suzanne Tilton, and Oksana Tsinman. In addition, I thank Dmytro Voloboy and Konstantin Tsinman for their help in database, computational, and theoretical matters. The helpful discussion with many colleagues, particularly Manfred Kansy and Holger Fischer at Hoffmann La-Roche, Ed Kerns and Li Di at Wyeth Pharmaceuticals, and those at Sirius Analytical Instruments, especially John Comer and Karl Box, are gratefully acknowledged. Helpful comments from Professors John Dearden (Liverpool John Moores University) and Hugo Kubinyi (Heidelberg University) are greatly appreciated. I also thank Professor Anatoly Belyustin (St. Peterburgh University) for pointing out some very relevant Russian literature. Chris Lipinski (Pfizer) has given me a lot of good advice over the last 10 years about instrumentation and pharmaceutical research, for which I am grateful. Collaborations with Professors Krisztina Takács-Novák (Semmelweis University, Budapest) and Per Artursson (Uppsala University) have been very rewarding. James McFarland (Reckon.Dat) and Alanas Petrauskas (Pharma Algorithms) have been my teachers of *in silico* methods. I am in debt to Professor Joan Abbott and Dr. David Begley for allowing me to spend three months in their

laboratory at King's College London, where I learned a lot about the blood–brain barrier. Omar at Cafe Minon, Warwick Street in Pimlico, London, was kind to let me spend many hours in his small sandwich shop, as I wrote several papers and drank a lot of coffee. Lasting thanks go to David Dyrssen and the late Jannik Bjerrum for planting the seeds of most interesting and resilient pH-metric methodologies, as well as to Professor Bernard Testa of Lausanne University for tirelessly fostering the white light of physicochemical profiling. My congratulations to him on the occasion of his retirement.

Alex Avdeef

Boston, Massachusetts
September 2002

ABBREVIATIONS

ABL (or UWL)	aqueous boundary layer (or unstirred water layer)
ADME	absorption, distribution, metabolism, excretion
AP	absorption potential
AS	anthroylstearic acid
AUC	area under the curve
BA/BE	bioavailability/bioequivalence
BBB	blood–brain barrier
BBM	brush-border membrane
BCS	Biopharmaceutics Classification System
BLM	black lipid membrane (single bilayer membrane barrier)
BMEC	brain microcapillary endothelial cell (*in vitro* cultured-cell model)
BPC	Brain Penetration Classification
BSA	bovine serum protein
CE	capillary electrophoresis
CGM	Classification Gradient Map
Cho	cholesterol
CL	cardiolipin
CMC	critical micelle concentration
CPC	centrifugal partition chromatography
CRE	Crone–Renkin equation
CV	cyclic voltammetry
DA	dodecylcarboxylic acid
DMPC	dimyristoylphosphatidylcholine
DOPC	dioleoylphosphatidylcholine

DRW	dynamic range window
DS	Double-Sink (PAMPA)
DSHA	*N*-Dansylhexadecylamine
DTT	Dissolution Template Titration (solubility method)
ECF	extracellular fluid (in the brain)
EMF	electromotive force (mV)
ER	efflux ratio (*in vitro* polarized transport)
ET	extrusion technique (for making LUV)
FAT	freeze-and-thaw (step in the making of LUV)
FDM	Facilitated Dissolution Method (solubility method)
FFA	free fatty acid
FLW	flow limit window
GIT	gastrointestinal tract
GOF	goodness-of-fit (in regression analysis)
HDM	hexadecane membrane
hERG	human ether-a-go-go related gene
HIA	human intestinal absorption
HJP	human jejunal permeability
HP-β-CD	2-hydroxypropyl-β-cyclodextrin
HTS	high-throughput screening or solubility
IAM	immobilized artificial membrane
ISF	interstitial fluid (in the brain)
IUPAC	International Union of Pure and Applied Chemistry
IVIVC	*in vitro–in vivo* correlation
KRB	Krebs–Ringer bicarbonate (buffer)
KO/WT	knockout/wild-type P-glycoprotein (Pgp)-transfected mouse models
LFER	linear free-energy relationship
LJP	liquid-junction potential (mV)
LOD	limit of detection
LUV	large unilamellar vesicle
M6G	morphine-6β-D-glucuronide
MAD	maximum absorbable dose (mg)
MBUA	mouse brain uptake assay
MDCK	Madin–Darby canine kidney (cell line)
MEP	molecular electronic potential
MLR	multiple linear regression
MLV	multilamellar vesicle
MSF	miniaturized shake-flask (solubility method)
NaTC	sodium taurocholate
NCE	new chemical entity
NIST (NBS)	National Institute of Standards and Technology (formerly known as the National Bureau of Standards, NBS)
NMP	1-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance

OECD	Organization for Economic Cooperation and Development
OIM	open innovation model (pharmaceutical industry collaborations)
OSYS	origin-shifted Yasuda–Shedlovsky (function in cosolvent pK_a analysis)
PA	phosphatidic acid
PAMPA	Parallel Artificial Membrane Permeability Assay
PAMPA–BBB	PAMPA used to predict blood–brain barrier permeability, based on PBLE formulation
PASS	partially automated solubility screen
PBLE	porcine brain lipid extract
PBPK	physiologically based PK
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PEG	polyethylene glycol oligomer
PG	phosphatidylglycerol or propylene glycol
PGDP	propylene glycol dipelargonate
PI	phosphatidylinositol
PK	pharmacokinetics
*p*OD	pK_a^{FLUX}—optimized design
PS	phosphatidylserine
PSA	polar surface area (*in silico* descriptor)
PVDF	polyvinylidene fluoride (hydrophobic filter membrane)
QSPR	quantitative structure–permeability relationship
RBC	red blood cell
SCFA	short-chain fatty acids
SIP	surface ion pair (charged-drug membrane surface partitioning)
SLS	sodium lauryl sulfate (anionic detergent)
RLJP	residual LJP
Sph	sphingomyelin
SSF	saturation shake-flask (solubility method)
SUV	small unilamellar vesicle
TEER	transendothelial electrical resistance ($\Omega\cdot cm^2$)
TJ	tight junction
TMA-DPH	trimethylamino-diphenylhexatriene chloride

NOMENCLATURE

A	area of the PAMPA filter (cm^2)
C_0	aqueous concentration of the uncharged species ($mol \cdot cm^{-3}$)
$C_m(x)$	solute concentration inside of a membrane, at position x ($mol \cdot cm^{-3}$)
C_m^x	solute concentration inside a membrane, at position x ($mol\ cm^{-3}$)
C_R, C_D	receiver and donor aqueous solute concentration, respectively ($mol \cdot cm^{-3}$)
D	Lipid–water distribution pH-dependent function (also called the apparent partition coefficient)
D_{aq} (D_m)	diffusivity of a solute in aqueous (membrane) solution ($cm^2 \cdot s^{-1}$)
diff	difference between the partition coefficient of the uncharged and the charged species
D_{MEM}	diffusivity of a solute inside a membrane ($cm^2 \cdot s^{-1}$)
$D_{MEM/W}$	pH-dependent membrane–water apparent partition coefficient (dimensionless)
Double-Sink	two sink conditions present: ionization and binding
$E(\Delta\varphi)$	function due to potential drop across the cell junction (dimensionless)
$f_{(0)}$, $f_{(+)}$, $f_{(-)}$	molecule concentration fraction in the uncharged, positively charged and negatively charged forms, respectively
$F(r_{HYD}/R)$	Renkin molecular sieving function, dimensionless fraction in the range of 0 to 1