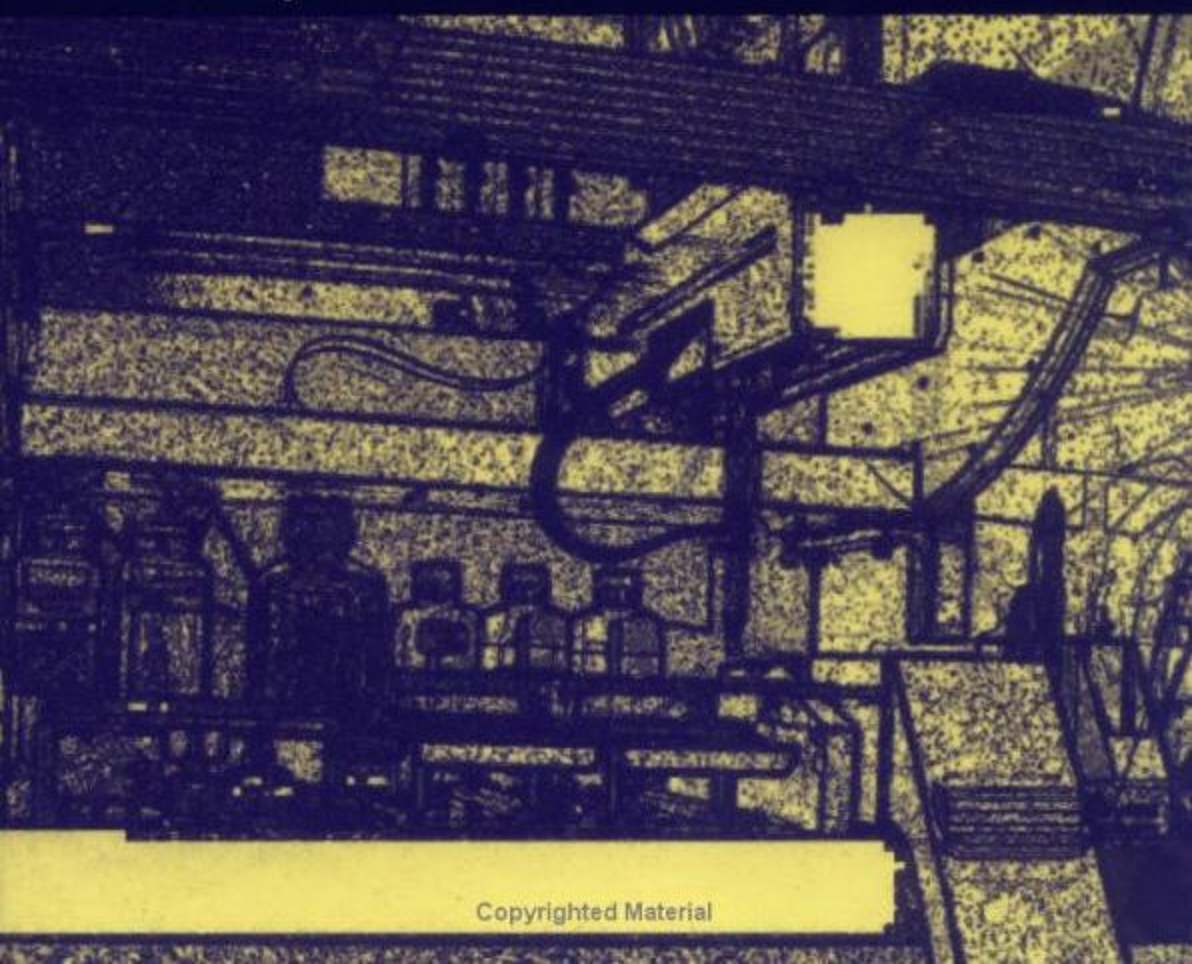
Absorption and Drug Development

Solubility, Permeability, and Charge State

Alex Avdeef



ABSORPTION AND DRUG DEVELOPMENT

ABSORPTION AND DRUG DEVELOPMENT

Solubility, Permeability, and Charge State

ALEX AVDEEF
*p*ION, Inc.

WILEY-INTERSCIENCE
A JOHN WILEY & SONS, INC., PUBLICATION

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.
Published simultaneously in Canada.

Library of Congress Cataloging-in-Publication Data:

Avdeef, Alex.
Absorption and drug development : solubility, permeability, and charge state / Alex Avdeef.
p. cm.
Includes index.
ISBN 0-471-42365-3 (Cloth)
1. Drugs–Design. 2. Drugs–Metabolism. 3. Drug development. 4. Absorption. I. Title.
RS420 .A935 2003
615′ .19–dc21 2003011397

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

Carla
Natalie
Michael

CONTENTS

PREFACE

This book is written for the practicing pharmaceutical scientist involved in absorption–distribution–metabolism–excretion (ADME) measurements who needs to communicate with medicinal chemists persuasively, so that newly synthesized molecules will be more ''drug-like.'' ADME is all about ''a day in the life of a drug molecule'' (absorption, distribution, metabolism, and 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 *parallel artificial membrane permeability assay* (PAMPA).

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

The author has observed that graduate programs in pharmaceutical sciences often neglect to adequately train students in these classical solution chemistry topics. Often young scientists in pharmaceutical companies are assigned the task of measuring some of these parameters in their projects. Most find the learning curve somewhat steep. Also, experienced scientists in midcareers encounter the topic of physicochemical profiling for the first time, and find few resources to draw on, outside 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 (postdoctoral fellow) in Professor Ken Raymond's group at the 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 5 years, more than 300 pages of hand-scribbled notes and derivations accumulated, but no book emerged. Some years later, a section 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* [112] out of the early effort in the startup 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 3-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 shared many new ideas during the courses. Since the early 1990s, 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 hightech highway 128, north of Boston, Massachusetts. The list of topics has expanded since 1990 to cover solubility, dissolution, and permeability, as new instruments were developed. In 2002, 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).'' [25] 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. For 3 months, I was privileged to join Professor Joan Abbott's neuroscience laboratory at King's College, London, 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 sum-P_e PAMPA GIT* model for predicting human 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 embedded 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 (pH), and lays the foundation for the rest of the book. Chapter 3 covers the topic of ionization constants—how to measure pK_a values accurately and quickly, and which methods to use. Bjerrum analysis is revealed as the ''secret weapon'' behind the most effective approaches. Chapter 4 discusses experimental

methods of measuring partition coefficients, log P and log D. It contains a description of the Dyrssen dual-phase potentiometric method, which 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 [473], has demonstrated capabilities to measure solubilities as low as 1 nanogram per milliliter (ng/mL). Also, 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 (parallel artificial membrane permeability assay), the high-throughput method introduced by Manfred Kansy et al. of Hoffmann-La Roche [547]. 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.

A. AVDEEF

ACKNOWLEDGMENTS

Professor Norman Ho (University of Utah) was very kind to critically read the Chapter 7 and comment on the various derivations and concepts of permeability. His unique expertise on the topic spans many decades. His thoughts and advice (30 pages of handwritten notes) inspired me to rewrite some of the sections in that chapter. I am very grateful to him. Special thanks go to Per Nielsen and Cynthia Berger of *p*ION for critically reading and commenting on the manuscript. I am grateful to other colleagues at *p*ION who expertly performed many of the measurements of solubility and permeability presented in the book: Chau Du, Jeffrey Ruell, Melissa Strafford, Suzanne Tilton, and Oksana Tsinman. Also, 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 Fisher 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 since 1992 on 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 3 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 place, 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, and 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.

DEFINITIONS

ACRONYMS*

AC	aminocoumarin
ADME	absorption, distribution, metabolism, excretion
ANS	anilinonaphthalenesulfonic acid
AUC	area under the curve
BA/BE	bioavailability–bioequivalence
BBB	blood–brain barrier
BBM	brush-border membrane
BBLM	brush-border lipid membrane
BCS	biopharmaceutics classification system
BLM	black lipid membrane
BSA	bovine serum albumin
CE	capillary electrophoresis
CHO	caroboxaldehyde
CMC	critical micelle concentration
CPC	centrifugal partition chromatography
CPZ	chlorpromazine
CTAB	cetyltrimethylammonium bromide
CV	cyclic votammetry
DA	dodecylcarboxylic acid
DOPC	dioleylphosphatidylcholine
DPPC	dipalmitoylphosphatidylcholine
DPPH	diphenylpicrylhydrazyl

DSHA	dansylhexadecylamine
DTT	dissolution template titration
EFA	evolving factor analysis
ET	extrusion technique (for preparing LUV)
FAT	freeze and thaw (step in LUV preparation)
FFA	free fatty acid
GIT	gastrointestinal tract
GMO	glycerol monooleate
HC	hydrocoumarin
HIA	human intestinal absorption
HJP	human jejunal permeability
HMW	high molecular weight
HTS	high-throughput screening
IAM	immobilized artificial membrane
IVIV	in vitro–in vivo
LUV	large unilamellar vesicle
MAD	maximum absorbable dose
MDCK	Madin–Darby canine kidney
MLV	multilamellar vesicle
M6G	morphine-6-glucuronide
NCE	new chemical entity
OD	optical density
PAMPA	parallel artifical membrane permeabillity assay
PC	phosphatidylcholine
PCA	principal-component analysis
PK	pharmacokinetic
QSPR	quantitative structure–property relationship
SCFA	short-chain fatty acid
SDES	sodium decyl sulfate
SDS	sodium dodecyl sulfate
SGA	spectral gradient analysis
SLS	sodium laurel sulfate
STS	sodium tetradecyl sulfate
SUV	small unilamellar vesicle
TFA	target factor analysis
TJ	tight junction
TMADPH	trimethylaminodiphylhexatriene chloride
UWL	unstirred water layer (adjacent to membrane surface)

NOMENCLATURE

C_A, C_D	aqueous solute concentrations on the acceptor and donor sides of a membrane, respectively (mol/cm^3)
C_0	aqueous concentration of the uncharged species (mol/cm^3)
C_m^x	solute concentration inside a membrane, at position x (mol/cm^3)
δ	difference between the liposome–water and octanol–water log P for the uncharged species
diff	difference between the partition coefficient of the uncharged and the charged species
Δ shift	the difference between the true pK_a and the apparent pK_a observed in a solubility–pH profile, due to DMSO–drug binding, or drug–drug aggregation binding
D_{aq}	diffusivity of a solute in aqueous solution (cm^2/s)
D_m	diffusivity of a solute inside a membrane (cm^2/s)
eggPC	egg phosphatidylcholine
h	membrane thickness (cm)
hit	a molecule with confirmed activity from a primary assay, a good profile in secondary assays, and with a confirmed structure
J	flux across a membrane (mol cm^{-2} s^{-1})
K_{sp}	solubility product (e.g., [Na^+][A^-] or [BH^+][Cl^-])
lead	a hit series for which the structure–activity relationship is shown and activity demonstrated in vivo
K_d or D	lipid–water distribution pH-dependent function (also called the ''apparent'' partition coefficient)
K_p or P	lipid–water pH-independent partition coefficient
K_e	extraction constant
$\bar{n}_{\text{H}}$	Bjerrum function: average number of bound protons on a molecule at a particular pH
P_a	apparent artificial-membrane permeability (cm/s)—similar to P_e, but with some limiting assumption
P_e	effective artificial-membrane permeability (cm/s)
P_m	artificial-membrane permeability (cm/s)—similar to P_e, but corrected for the UWL
P_0	intrinsic artificial-membrane permeability (cm/s), that of the uncharged form of the drug
pH	operational pH scale
p$_c$H	pH scale based on hydrogen ion concentration
pK_a	ionization constant (negative log form), based on the concentration scale
p$_o K_a$	apparent ionization constant in an octanol–water titration
pK_a^{oct}	octanol pK_a (the limiting p$_o K_a$ in titrations with very high octanol–water volume ratios)
pK_a^{mem}	membrane pK_a

pK_a^{gibbs}	ionization constant corresponding to the pH at which both the uncharged and the salt form of a substance coprecipitate
pK_a^{flux}	apparent ionization constant in a log P_e–pH profile, shifted from the thermodynamic value as a consequence of the unstirred water layer; the pH where 50% of the resistance to transport is due to the UWL and 50% is due to the lipid membrane
sink	any process that can significantly lower the concentration of the neutral form of the sample molecule in the acceptor compartment; examples include physical sink (where the buffer solution in the acceptor compartment is frequently refreshed), ionization sink (where the concentration of the neutral form of the drug is diminished as a result of ionization), and binding sink (where the concentration of the neutral form of the drug is diminished because of binding with serum protein, cyclodextrin, or surfactants in the acceptor compartment)
double-sink	two sink conditions present: ionization and binding
S	solubility in molar, μg/mL, or mg/mL units
S_i	solubility of the ionized species (salt), a conditional constant, depending on the concentration of the counterion in solution
S_0	intrinsic solubility, that is, the solubility of the uncharged species
τ_{LAG}	the time for steady state to be reached in a permeation cell, after sample is introduced into the donor compartment; in the PAMPA model described in the book, this is approximated as the time that sample first appears detected in the acceptor well