Lipophilicity in Drug Action and Toxicology

edited by
Vladimir Pliška
Bernard Testa
Han van de Waterbeemd

Weinheim · New York · Basel · Cambridge · Tokyo

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Series Editors:
Prof. Dr. Raimund Mannhold
Biomedical Research Center
Molecular Drug Research Group
Heinrich-Heine-Universität
Universitätsstraße 1
D-40225 Düsseldorf
Germany

Prof. Dr. Hugo Kubinyi
ZHV/W, A 30
BASF AG
D-67056 Ludwigshafen
Germany

Prof. Dr. Hendrik Timmerman
Faculty of Chemistry
Dept. of Pharmacochemistry
Free University of Amsterdam
De Boelelaan 1083
NL-1081 HV Amsterdam
The Netherlands

Volume editors:
Prof. Dr. Vladimir Pliška
Department of Animal Science
Swiss Federal Institute of
Technology – ETH Zürich
Tannenstr. 1
CH-8092 Zürich
Switzerland

Prof. Dr. Bernard Testa
University of Lausanne
School of Pharmacy
CH-1005 Lausanne
Switzerland

Priv.-Doz. Dr. Han van de Waterbeemd
Hoffman-La Roche Ltd.
Pharma Research New Technologies
CH-4002 Basel
Switzerland

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Preface

After three volumes covering the various techniques quantifying the relations of biological activity and chemical properties of drug molecules, the fourth volume in the series "Methods and Principles in Medicinal Chemistry" focuses on the role of lipophilicity in drug action and toxicology.

Lipophilicity is well known as a prime physico-chemical descriptor of xenobiotics with relevance to their biological properties. The hydrophobic interactions of drugs with their receptors, the pharmacokinetic behaviour of drug molecules, toxicological properties and pharmaceutical aspects like solubility are examples of a steadily increasing number of topics in which lipophilicity plays an important role.

In keeping with the outstanding importance of lipophilicity in biosciences, this topic is treated in the present volume by more than twenty leading experts. The first out of five sections covers the physico-chemical background of molecular interactions and partitioning. The following two sections deal with the various experimental and computational approaches to quantifying lipophilicity. Experimental assessment includes partitioning as well as chromatographic alternatives. Computational procedures range from the classical approach employing hydrophobic fragmental constants to three-dimensional concepts which reflect the impact of conformational aspects of lipophilic behaviour. The last two sections reflect the relevance of lipophilicity in biological responses to xenobiotics and in drug design. Inter alia, the dependence of pharmacokinetic processes, like membrane transport and biotransformation on lipophilicity as well as environmental hazard assessment using lipophilicity data, deserve mention here. Lipophilicity scales for peptides and amino acids are discussed in their relation to drug design.

The present volume convincingly achieves its main objective, to put emphasis on lipopilicity as an important property for a vast number of biological processes.

December 1995

Düsseldorf — Raimund Mannhold
Ludwigshafen — Hugo Kubinyi
Amsterdam — Hendrik Timmerman

Methods and Principles in Medicinal Chemistry

Edited by
R. Mannhold
H. Kubinyi
H. Timmerman

Volume 1
Hugo Kubinyi
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Volume 2
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Volume 3
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Advanced Computer-Assisted Techniques in Drug Discovery

Volume 4
Vladimir Pliška, Bernard Testa, Han van de Waterbeemd (eds.)
Lipophilicity in Drug Action and Toxicology

Volume 5
Hans-Dieter Höltje, Gert Folkers
Molecular Modeling

A Personal Foreword

The idea to write a state-of-the-art monograph examining manifold aspects of lipophilicity was born at the 7th European QSAR Symposium in Interlaken (1988). The Organizing Committee suggested that the spared funds of the Symposium be utilized for organizing a workshop on lipophilicity which would establish a solid base for such a monograph, and challenged us to undertake the task. It took us six long years to fulfill the first part of our commitment. The *Symposium on Lipophilicity in Drug Research and Toxicology* was held in Lausanne in March 1995, and was only possible thanks to an additional support by numerous companies and organizations. The remaining task turned out to be even more difficult. To publish a book *so* specified meant to ask a number of authors for collaboration. The our great joy, the majority of the contacted persons accepted, wrote their chapters, and even delivered their manuscript in time. We thank them for their collaboration.

However, editing is a thankless task. The text of any book of this series should be generally comprehensible, thus assuming a more or less consistent style. In trying this, editors are put under pressure by both authors and publisher, for different reasons. On top of it, there are also series editors who have firm and generally justified notion about the style of the entire book series. There is also a different degree of bias by individual partners: in our estimate the highest one with the authors, who usually present their favorite child with a great deal of an understandable enthusiasm, and the lowest one with the publishers who ist obliged to consider – and to foresee – the general success of the publication. Editors and series editors may find themselves somewhere half-way in between. Provided that the final outcome does *not* have a character of conference proceedings, the editors are compelled to set up basic style rules, and to exercise a certain pressure on the authors to follow them. In most instances, this was benevolently understood and respected.

The book contains, besides purely methodological contributions and established physico-chemical concepts, also chapters which my seemingly touch the problem of lipophilicity only from afar, or which may rather be considered as dreams of the future. However, we are conviced that they have a rightful place in this book.

There are many persons, in additions to the participating authors, to whom we owe our thanks. To name only very few of them, Professor Jean-Luc Fauchère gave the spiritual, and also material, impulse to this book. The VCH editors, Dr. Thomas Mager and Dr. Michael Bär carried out all the burdens asociated with the preparation for printing and manufacturing. And our colleagues, the series editors Professors Raimund Mannhold, Hugo Kubinyi, and Hendrik Timmerman, were most helpful with their critical comments.

February 1996

Zürich — Vladimir Pliška
Lausanne — Bernard Testa
Basel — Han van de Waterbeemd

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List of Contributors

Michael H. Abraham
The Department of Chemistry
University College London
20 Gordon Street
London WC1H 0AJ
UK

Alex Avdeef
Sirius Analytical Instruments Ltd.
Riverside
Forest Row Business Park
Forest Row
East Sussex RH18 5DW
UK

Štefan Baláz
Department of Biochemical Technology
Slovak Technical University
Radlinského 9
SK-81237 Bratislava
Slovakia

Frédéric Billois
Institut de Chimie Therapeutique
BEP
Université de Lausanne
CH-1015 Lausanne-Dorigny
Switzerland

Ronald T. Borchardt
Pharmaceutical Chemistry Department
University of Kansas
Lawrence, KS
USA

Philip S. Burton
Drug Delivery Research Systems
Upjohn Laboratories
Kalamazoo, MI
USA

Pierre-Alain Carrupt
Institut de Chimie Thérapeutique
BEP
Université de Lausanne
CH-1015 Lausanne-Dorigny
Switzerland

Harpreet S. Chadha
The Department of Chemistry
University College London
20 Gordon Street
London WC1H 0AJ
UK

Marvin Charton
Chemistry Department
Pratt Institute
Brooklyn, NY 11205
USA

Chao-Kun Cheng
Department of Mathematical Sciences
Virginia Commonwealth University
Richmond, VA
USA

Robert A. Conradi
Drug Delivery Research Systems
Upjohn laboratories
Kalamazoo, MI
USA

Karl Dross
C. and O. Vogt Institute for Brain Research
Heinrich-Heine-Universität
Universitätstrasse 1
40225 Düsseldorf
Germany

Emanuel Escher
Département de Pharmacologie
Faculté de Médecine
Université de Sherbrooke
Sherbrooke (Quebec)
J1H 5N4
Canada

Jean-Luc Fauchère
Institut de Recherches SERVIER
11 rue des Moulineaux
92150 Suresnes
France

Holger Fischer
F. Hoffmann-La Roche Ltd.
Pharma Research New Technologies
Structure-Property Correlations Group
CH-4002 Basel
Switzerland

Gerd Folkers
Department of Pharmacy
ETH Zürich
Winterthurerstrasse 190
CH-8057 Zürich
Switzerland

Patrick Guillard
Institut de Chimie thérapeutique
BEP
Université de Lausanne
CH-1015 Lausanne-Dorigny
Switzerland

Pavel Hobza
J. Heyrovský Institute of Physical Chemistry
Academy of Sciences of the Czech Republic
Dolejškova 3
182 23 Prague 8
Czech Republic

Manfred Kansy
F. Hoffmann-La Roche Ltd.
Pharma Research New Technologies
Structure-Property Correlations Group
CH-4002 Basel
Switzerland

Lemont B. Kier
Department of Medicinal Chemistry
Virginia Commonwealth University
Richmond, VA
USA

Albert J. Leo
Pomona College and BioByte Corporation
Claremont
California, CA 91711–0517
USA

Robert L. Lipnick
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, DC 20460
USA

Giuseppe Lisa
Institut de Chimie Thérapeutique
BEP
Université de Lausanne
CH-1015 Lausanne-Dorigny
Switzerland

Raimund Mannhold
Department of Laser Medicine
Molecular Drug Research Group
Heinrich-Heine-Universität
Universitätstrasse 1
D-40225 Düsseldorf
Germany

Alfred Merz
Department of Pharmacy
ETH Zürich
Winterthurerstrasse 190
CH-8057 Zürich
Switzerland

Christophe Meyer
Institut National de la Recherche Agronomique
BP 527
F-44026 Nantes Cedex
France

Serge Pérez
Institut National de la Recherche Agronomique
BP 527
F-44026 Nantes Cedex
France

Vladimir Pliška
Department of Animal Science
Swiss Federal Institute of Technology
ETH Zürich
CH-8092 Zürich
Switzerland

W. Graham Richards
Physical and Theoretical Chemistry
Oxford University
Oxford
UK

Christoph Sonntag
C. and O. Vogt Institute for Brain Research
Heinrich-Heine-Universität
Universitätstrasse 1
D-40225 Düsseldorf
Germany

Albert Taylor
Servier Research and Development
Fulmer Hall
Windmill Road
Fulmer
Slough SL3 6HH
UK

Bernard Testa
Institut de Chimie Thérapeutique
BEP
Université de Lausanne
CH-1015 Lausanne-Dorigny
Switzerland

Ruey-Shiuan Tsai
Institut de Chimie Thérapeutique
BEP
Université de Lausanne
CH-1015 Lausanne-Dorigny
Switzerland

Michael S. Tute
Chemistry Department
University of Kent at Canterbury
Canterbury
Kent
UK

Peter Vis
Servier Research and Development
Fulmer Hall
Windmill Road
Fulmer
Slough SL3 6HH
UK

Björn Wagner
F. Hoffmann-La Roche Ltd.
Pharma Research New Technologies
Structure-Property Correlations Group
CH-4002 Basel
Switzerland

Bernard Walther
Servier Research and Development
Fulmer Hall
Windmill Road
Fulmer
Slough SL3 6HH
UK

Han van de Waterbeemd
F. Hoffmann-La Roche Ltd.
Pharma Research New Technologies
Structure-Property Correlations Group
CH-4002 Basel
Switzerland

Peter Weber
Institut de Chimie Thérapeutique
BEP
Université de Lausanne
CH-1015 Lausanne-Dorigny
Switzerland

Rudolf Zahradník
J. Heyrovský Institute of Physical Chemistry
Academy of Sciences of the Czech Republic
Dolejškova 3
182 23 Prague 8
Czech Republic

Contents

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1 Lipophilicity: The Empirical Tool and the Fundamental Objective. An Introduction

Vladimir Pliška, Bernard Testa and Han van de Waterbeemd

1.1 Setting the Scene

At the end of the 7th QSAR Symposium held in Interlaken in 1988, the organisers asked a number of participants which topics they felt should require greater attention in future meetings. The list of suggestions was indeed long and diverse. One subject, however, was mentioned almost unanimously, namely the pharmacological, toxicological, and pharmacokinetic significance of weak interactions in general and lipophilicity in particular.

This interest is understandable and legitimate. Weak interactions such as hydrogen bonds, van der Waals forces, hydrophobic effects, and charge transfer interactions are absolutely essential for molecular recognition and interactions in living systems. They underlie the formation of firmly determined molecular and supramolecular structures (for instance in biological macromolecules, membranes, etc.) and, at the same time, enable their amazing flexibility and adaptability. As a rule, several weak forces participate in any interaction occurring in a biological system. Due to their superposition, intermolecular and intramolecular complexes may exhibit a broad range of association constants from about 10^4 mol L^{-1} (enzyme-substrate complexes) to 10^{14} mol L^{-1} (polyvalent antibody-antigen complexes). Since biologically important macromolecules always contain a variety of polar and nonpolar sites, the role of polar and hydrophobic forces is of utmost significance in all processes of biological recognition.

Before going any further, it appears appropriate to comment on the words "hydrophobicity" and "lipophilicity" since they are used rather loosely and inconsistently in the literature. As discussed in greater details below, lipophilicity is a molecular property expressing the relative affinity of solutes for an aqueous phase and an organic, water-immiscible solvent. As such, lipophilicity encodes most of the intermolecular forces that can take place between a solute and a solvent. In contrast, hydrophobicity is a consequence of attractive forces between nonpolar groups (e.g., hydrocarbon chains and rings) and therefore is but one component of lipophilicity. Factorization of lipophilicity into its polar and hydrophobic components contributes considerably to our understanding of the nature of lipophilicity and its role in the biological world [1, 2].

1.2 Biological Aspects

The relation between lipid solubility and biological effects of drugs was recognized almost a century ago by Meyer [3] and by Overton [4]. Some decades later, Pauling discovered a relationship between lipophilicity and anesthetic potency in a series of chem-

ically heterogeneous compounds [5]. It soon became evident that a quantification, or even a description of lipophilicity in thermodynamic terms, is not practicable. Until now, only empirical scales of lipophilicity have been of importance in practice, some expressing the changes in free energy associated with solute transfer between two phases, others being dimensionless indices relating partitioning data of given solutes to a general standard. This latter approach is based on the assumption of linear free-energy changes and is represented by the Leffler-Grunwald operators [6]. It was, in fact, first employed by Hammett in 1935 to describe electronic properties of substituent groups attached to a fixed molecular backbone [7]. Later, Zahradnik and coworkers used responses obtained in two related biological systems to derive what is in fact, but not by name, a set of lipophilicity constants [8, 9]. Such attempts were not unique during the late 1950s and early 1960s. However, it is to the great credit of Hansch, Fujita and Leo that empirical constants can be readily used in pharmacology and toxicology [10, 11]. Besides deriving an extensive set of lipophilicity descriptors, the so-called π-values, Hansch and colleagues proved their apparent additive nature, thus establishing them as genuine substituent constants.

The structure and function of any biological system are closely related to the lipophilic properties of its component molecules. First, lipid-lipid interactions strongly influence the structure of biological membranes, and thereby the compartmentation of compounds within cell organelles. Second, transport and distribution processes within biological systems are to a large extent controlled by the lipophilicity of the system components. The highly hydrophobic interior of a bilayer membrane enables or facilitates the passage of lipophilic substances and prevents the free diffusion of polar molecules except water in and out of cells and organelles. By controlling both transport and compartmentation processes with some degree of selectivity, lipophilicity imposes an adjustable resistance to free diffusion, thus becoming the major obstacle to a random distribution of substances in biological systems, which would be entirely incompatible with life. The same is true for distribution within an organism where several physiological barriers control the access of endogenous and exogenous compounds to various organs and tissues. It is well established that the hemato-encephalic (blood-brain), placental and hemato-mammary (blood-mammary gland) barriers are of a very selective nature, so that specific transporter systems have to mediate the passage of vital compounds, the hydrophilicity of which prevents their passive membrane permeation.

Last, but not least, lipophilicity plays a dominating role in ligand-receptor interactions, e.g., in the binding of hormones, neurotransmitters, modifiers of cellular processes (e.g., growth, initiation, or repression factors) and drugs to their receptors. The same applies for enzyme-substrate, enzyme-inhibitor, antigen-antibody and other ligand-macromolecule interactions.

1.3 The Molecule in the Background

While molecular pharmacology deals with the response of a cell to a substance recognized as a message, medicinal chemistry attempts to unveil the semantics, and perhaps also the syntax, of the molecular language which encodes these messages. In order to achieve this, molecular structure has to be described in a pharmacologically relevant

way; adoption of a multilevel description of molecular structure [12] appears to be the best approach to this end. Such a description starts at a simple geometrical level, continues with a stereoelectronic one, and ends up at levels of intermolecular interactions. It is at these latter levels that one encounters properties like solubility and lipophilicity whose high content in structural information remains difficult to understand fully.

Lipophilicity, however, is far from being only an empirical tool in structure-activity analysis. It is also a unique probe that can be used to unravel the complex and dynamic interplay between intermolecular forces and intramolecular interactions in solutes of interest. The former comprise interactions between a solute and the aqueous and organic phases, namely [1, 2, 13]:

- Ion-ion and ion-dipole (permanent, induced) interactions (for ionic solutes);
- Charge transfer interactions;
- Hydrogen bonds (normal, reinforced);
- Van der Waals interactions (forces of orientation, induction, and dispersion);
- Hydrophobic bonds.

Intramolecular interactions that influence lipophilicity can be classified as follows:

- Through-bond electronic effects a) in aromatic systems, and b) across aliphatic segments;
- Through-space electronic/polar effects comprising a) internal electrostatic bonds (ionic bonds, H-bonds, and other electrostatic bonds), b) internal electrostatic repulsion, and c) collision of hydration spheres due to proximity effects between polar groups;
- Through-space steric/hydrophobic effects comprising a) internal hydrophobic bonds (hydrophobic collapse), and b) internal steric hindrance.

Intramolecular interactions can explain differences in lipophilicity seen between regioisomers and between configurational diastereomers. The interplay between conformational diastereomerism and lipophilicity, which is particularly manifest in molecular chameleons, is gaining increasing recognition in compounds of sufficient size and functional complexity [14]. Furthermore, intramolecular interactions affecting lipophilicity represent a major and incompletely understood challenge to the accuracy of current fragmental systems.

1.4 Some Pragmatic Aspects

1.4.1 Definitions and Symbols

At this point, we should make an explanatory comment concerning the expressions "hydrophobicity" and "lipophilicity". Their usage is not uniform. Semantically, they seem to stand for the same feature or object, and are therefore frequently considered to be synonymous. In the scientific use, however, their meaning is quite different. The following operational definitions have been suggested by the IUPAC [15, 16]:

- **Hydrophobicity** is the association of nonpolar groups or molecules in an aqueous environment which arises from the tendency of water to exclude nonpolar molecules.

- **Lipophilicity** represents the affinity of a molecule or a moiety for a lipophilic environment. It is commonly measured by its distribution behavior in a biphasic system, either liquid-liquid (e.g., partition coefficient in 1-octanol/water) or solid-liquid (e.g. retention on RP-HPLC or TLC, see section 1.4.2) systems.

Such definitions are by no means unambiguous and noncontroversial, as our knowledge of molecular mechanisms underlying these phenomena, although continuously growing, is still far from being complete. There are, however, pragmatic reasons for their (albeit tentative) differentiation, and it is therefore not astonishing that they occur with different frequency in languages used in different research disciplines. The term "hydrophobicity" is familiar to biophysicists working with X-ray diffraction, NMR spectroscopy and molecular models. It is used in connection with the description of the molecular surface of a compound in contact with an aqueous environment. "Lipophilicity" is a term mainly employed by medicinal chemists to describe transport processes of a compound in biological systems. Much confusion also exists in the symbols of lipophilicity parameters. To bring some clarity, we offer in Table 1 a compilation of useful symbols.

1.4.2 Experimental Techniques

A great step forward has been achieved since the pioneering work of Meyer [3] and of Overton on the partitioning of anesthetics in olive oil/water [4]. Hansch, Fujita and their coworkers chose the 1-octanol/water solvent system as an arbitrary standard for expression of lipophilicity [10, 11], and pioneered its measurement by the shake-flask technique. Most of the available data refer to this partitioning system. This standard technique, sometimes laborious and precarious, can efficiently and quite safely be sub-

Table 1. Lipophilicity parameters and their recommended symbols

Referred Symbol	Parameter	Alternatives
log P	partition coefficient of neutral species[a]	log K, log PC
log P_{oct}	log P for 1-octanol/water	
log P_{alk}	log P for alkane/water	
log D	distribution coefficient: "apparent" partition coefficient[a]	log P', log P_{app}
log D_{oct}	log D for 1-octanol/water	
log D_{alk}	log D for alkane/water	
log P^+	partition coefficient of cationic form	
log P^-	partition coefficient of anionic form	
log $P^{+/-}$	partition coefficient of zwitterionic form	
CLOGP	log P calculated by the CLOGP program[b]	
log k	log of capacity factor in RP-HPLC	
log k_w	log k extrapolated to 100 % aqueous eluent	

[a] log P and log D can be calculated one from the other using the appropriate correction for ionization [1].
[b] Reference [19].

stituted by various chromatographic techniques: thin-layer chromatography (TLC), reversed-phase high performance liquid chromatography (RP-HPLC or RPLC), and centrifugal partition chromatography (CPC), which are all employed routinely.

1.4.3 Computational Procedures

Lipophilicity has been expressed by means of manifold descriptors mainly based on partition coefficients or similar thermodynamic features. Relationships between individual scales are, apart from some exceptions, very close. These descriptors can be obtained by a number of computational routines; they receive attention in this book. Fragmental constants, i.e., contributions of individual molecular fragments to the overall value of a descriptor, are roughly additive and thus afford quick predictions of lipophilicity from molecular structures. In this way, some problems and limitations associated with the experimental assessment of substituent constants can be overcome [17]. From a visual point of view, this property has been simulated as a dynamic process, exhibiting the characteristics observed experimentally [18].

From a practical point of view, lipophilicity descriptors are important for at least two reasons. First, they may predict unsatisfactory drug candidates and avoid, in a simple way, an extensive experimentation. This relates to both transport properties and intrinsic activity of the potentially interesting substances. Second, they enable to investigate structure-property relationships, in particular intermolecular forces and intramolecular interactions. These relationships are of utmost importance in drug design. It would be, for example, of little use to design a highly hydrophilic substance if it is targeted to the central nervous system.

1.5 Objectives of the Book

In summary, lipophilicity is an essential property of molecules whose roles in biological systems are numerous and essential. Above all, it is intimately connected with regulatory pathways in living systems, and allows them to exist away from equilibrium. In so far as medicinal chemists and pharmacologists aim at sending messages (i.e., drugs) to ailing cells, they cannot avoid viewing lipophilicity as one of the most significant properties controlling both the delivery and the reception of the message.

The aim of this monograph is therefore rather straightforward, namely, to present the state-of-the-art of the area, to bring about a current insight necessary for interpretation of lipophilicity data, and to demonstrate how research in cell biology, pharmacology, medicinal chemistry, toxicology, and related fields can benefit from them. Our main interest, however, is to put emphasis on lipophilicity as an important property controlling a great many processes in living organisms.

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2 Lipophilicity: A History

Michael S. Tute

Abbreviations

AM1	Molecular orbital program (Dewar)
CLOGP	Program for lipophilicity calculation
HPLC	High performance liquid chromatography
HYDRO	Program for lipophilicity calculation on flexible solutes
ISA	Isotropic (non-polar) surface area
SASA	Solvent accessible surface area
SCAP	Program for lipophilicity calculation using solvent-dependent conformations
TLC	Thin layer chromatography

Symbols

D	Distribution coefficient
f	Hydrophobic fragmental constant
P	Partition coefficient, refers usually to octanol/water
S	Molar solubility
S_A	Surface area
S_i	Atomic surface area
T_m	Melting point
V	Solute volume
α_H	Solute H-bond donor strength
β_H	Solute H-bond acceptor strength
Δq_i	Atomic partial charge
π	Hydrophobic substituent constant
π^*	Solute polarity/polarisability
π_H	Enthalpic component of hydrophobic substituent constant
π_s	Entropic component of hydrophobic substituent constant
σ	Hammett electronic substituent constant

2.1 Introduction

Lipophilicity is usually expressed by the partition coefficient (log$P$), a molecular parameter which describes the partitioning equilibrium of a solute molecule between water and an immiscible lipid-like organic solvent. By convention, the ratio of concentrations in the two phases is given with the organic phase as numerator, so that a positive value for log$P$ reflects a preference for the lipid phase, and a negative value reflects a

relative affinity for water. Also by convention, where ionizable molecules are concerned, log$P$ refers to the neutral species whereas what is actually measured may be the distribution coefficient, log$D$. The distribution coefficient refers to the ratio of total concentrations of ionized and unionized species across both phases.

Many workers have emphasized that the value of log$P$ depends largely on interactions made by the solute with the water phase, either being repelled by water (hydrophobic effect) or solvated by water through hydrogen bonds or other polar forces (hydrophilic effect). Such emphasis has encouraged use of the term hydrophobicity, and in medicinal chemistry and particularly for QSAR the substituent constants, π, and fragment constants, f, are almost universally described as hydrophobic substituent parameters or hydrophobic fragmental constants.

Use of the term hydrophobicity has also been dependent on a perception of the thermodynamics of partitioning of strictly nonpolar solutes such as the aliphatic and aromatic hydrocarbons between water and a lipid phase, and on a particular use of the term "hydrophobic bonding" to describe the tendency of nonpolar groups to associate in aqueous solution, thereby reducing the extent of contact with neighboring water molecules. As discussed by Némethy [1], the formation of such "hydrophobic bonds" has long been considered to be driven by an entropy effect: the water molecules become more ordered around exposed nonpolar residues, and when the hydrophobic "bond" is formed, the order decreases, resulting in a favorable entropy and hence free energy of formation. For over 30 years, it has been commonly supposed that the "hydrophobic" interaction between nonpolar side chains of a protein, associated with formation and breakdown of layers of abnormal water, makes a prominent contribution to the stability of the native, folded form. The existence, nature, and effect of "hydrophobic hydration" is today a subject of intense controversy (see sction 2.4.2).

Use of the term hydrophobicity by the Hansch group [2], who in 1964 pioneered the use of octanol/water as the standard solvent pair for measurement, could also be justified on the grounds that this particular solvent pair is such that polar effects are similar in each phase. Both water and octanol have hydroxyl groups that can participate in polar interactions with the solute molecule, and moreover there is a considerable amount of water within the octanol phase. So, an octanol/water log$P$ value will emphasize differences in hydrocarbon interactions with water and with lipid, but tend to hide differences in the interaction of polar and hydrogen-bonding groups.

Recent studies have clearly and repeatedly shown that log$P$ in general incorporates two major contributions, namely a "bulk" term reflecting both hydrophobic (entropic) and dispersion (enthalpic) effects, and electrostatic terms reflecting hydrogen bonds and other dipole-dipole effects. Moreover, the emphasis on interaction of the solute with the water phase has been challenged; more emphasis has now been placed on enthalpic interactions within the lipid phase; free energy simulations have been carried out and thermodynamic measurements have been made to better understand fundamental interactions of the solute with each phase. As a result, traditional explanations of partitioning in terms of "hydrophobic bonding" have had to be reconsidered.

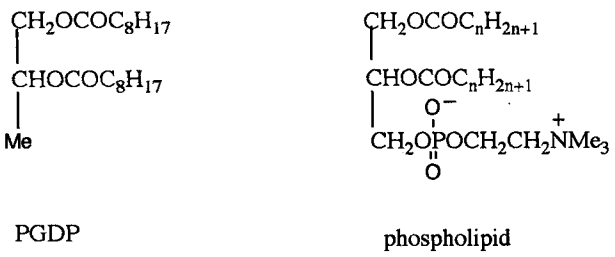
2.2 Measurement of Lipophilicity

The partition coefficient was first defined in 1872 by Berthelot and Jungfleisch [3], who wrote *"On the Laws that Operate for the Partition of a Substance between two Solvents"*. It was first used to correlate and explain the potencies of biologically active substances at the turn of the century, by both Meyer [4] and Overton [5] in their studies of narcotic compounds. Overton's work stimulated other investigations of the use of partition coefficients for biological correlations, among them a study by Seidell [6] in 1912. Believing that the partition coefficient of thymol might be relevant to a study of the mode of action of thymol against hookworm, Seidell made measurements using a variety of lipid phases, including olive oil, castor oil, peanut oil, and linseed oil. In those days, measurement was particularly tedious: it was necessary to separate thymol from the oil by a steam distillation, and then to estimate thymol in water by treatment with bromine, titrating the resulting hydrobromic acid produced!

With the development of UV spectroscopy, measurement of the partition coefficient for compounds with strong absorption, nonextreme values, and sufficient solubility in the aqueous phase has become routine, using the "shake-flask" method, partitioning between one of a wide variety of lipid phases, and water or an appropriate buffer solution as the aqueous phase. For many ionizable compounds, compounds of low solubility, and compounds with low UV absorbance or extreme values of partition coefficient then special methods of measurement or alternative lipophilicity parameters have had to be devised.

In 1959, Gaudette and Brodie [7] realized both the possibility for using a partition coefficient to model lipophilic character, and the relevance of lipophilicity to pharmacokinetic processes. They found a parallel between the heptane/buffer partition coefficients of certain drugs, and their rate of entry into cerebrospinal fluid. However, generalised use of log$P$ as a lipophilicity parameter did not come about until after 1964, with the Hansch octanol/water system remaining to this day the standard for both experimental and theoretical investigations. In 1971, Leo, Hansch and Elkins [8] published the first comprehensive review of partition coefficients, with a tabulation of nearly 6000 values, including their own measurements on some 800 in the octanol/water system. The review incorporated an account of the shake-flask method of measurement, which was discussed more exhaustively in a 1973 monograph by Purcell, Bass and Clayton [9].

Octanol/water log$P$ has also been measured by high-performance liquid chromatography [10], and by using a filter-probe to sample selectively from the aqueous or lipid phase so there is no need to fully separate the phases [11, 12]. For ionizable compounds, Brandström in 1963 [13] was first to use a potentiometric titration technique. One aqueous phase titration, with a pH-meter probe, was carried out in the aqueous phase to determine pK_a. A second titration was carried out in the presence of octanol, when partition occurred and the pK_a shifted. The difference in pK_a was related to log$P$. In 1974 Seiler [14] modified this technique so as to determine pK_a and log$P$ from a single titration. The technique has now been refined to enable not only simultaneous pK_a and log$P$ determination, but to allow treatment of substances with multiple ionization constants, ion-pair partitioning, and self-association reactions leading to the formation of oligomers [15, 16].



PGDP phospholipid

Figure 1. Structural similarities between propylene glycol dipelargonate (PGDP) and a phospholipid molecule.

Lipophilicity has, since 1964, been traditionally measured in the octanol/water system. However, for particular purposes and for particular sets of compounds, other solvent pairs have been used. Octanol/water values have been shown to be generally satisfactory for modeling serum protein binding and for modeling lipophilic interactions with biological membranes consisting largely of protein, but for other types of membrane then a different solvent system might be more appropriate. In 1989, Leahy et al. [17] suggested that membranes (or receptors) could exist with very different hydrogen bonding characteristics from those of octanol. Thus, membranes may contain neither acceptors nor donors (modeled by an alkane); or contain largely amphiprotic groups (as in a protein, modeled by octanol); largely proton donor groups (which may be modeled by chloroform); or largely proton acceptor groups (as in a phospholipid membrane). Leahy argued for the use of propylene glycol dipelargonate (PGDP) as lipid phase to model phospholipid membranes (Fig. 1) and have accordingly measured many partition coefficients in the PGDP/water system [18].

For many compounds, the traditional equilibrium method of partition coefficient measurement may be impossible, impractical, or inappropriate. As a practical alternative to log$P$, particularly for biological correlations, much use has been made of parameters derived from chromatographic retention. In 1941 Martin and Synge [19] showed that for reversed phase thin-layer chromatography, Eq. (1) relates partition coefficient, P, to the ratio, R_f, of distances moved by the compound spot and the solvent front in a given time, with K being a constant for the system. In 1950 Bate-Smith and Westall [20] defined the parameter R_m as in Eq. (2) from which Eq. (3) follows. In practice, excellent correlations have been found between R_m and log$P$ taking the form of Eq. (4). Kaliszan [21] has reviewed the use of lipophilicity parameters derived from HPLC, TLC, and paper chromatography.

$$P = K\left[\left(\frac{1}{R_f}\right) - 1\right] \tag{1}$$

$$R_{\mathrm{m}} = \log\left[\left(\frac{1}{R_f}\right) - 1\right] \tag{2}$$

$$R_{\mathrm{m}} = \log P - \log K \tag{3}$$

$$R_{\mathrm{m}} = a \log P + b \tag{4}$$

2.3 Calculation of Lipophilicity

2.3.1 Substitution Method

The Hansch group were the first to point out [2] in their influential paper of 1964, that the octanol/water log$P$ value of simple benzenoid derivatives could be calculated by a method bearing close analogy to the Hammett [22] treatment of chemical reactivity, including ionization, of substituted benzene derivatives. Hammett had shown in the 1930s that the equilibrium or rate constant of parent (unsubstituted) molecule, K_H, and the equilibrium or rate constant for a substituted compound, K_X, could be correlated by

$$\log\left(\frac{K_X}{K_H}\right) = \varrho\sigma_X \tag{5}$$

which could be rewritten as

$$\log K = \varrho\sigma_X + \log K_H \tag{6}$$

The substituent constant σ_X refers to the electronic effect of the substituent and is a parameter applicable to many different reactions (characterized by different values of ϱ) whose rate depends on the degree of electron release or withdrawal by the substituent. For the derivation of σ constants, the ionization of benzoic acids was defined as the standard reaction for which ϱ was set to unity. In analogous fashion to the Hammett treatment, Hansch defined substituent constants, π, by Eq. (7), choosing octanol/water as the standard system. Then, by analogy to Eq. (6) for reaction rates or equilibria, Eq. (8) could be used to calculate log$P$.

$$\log\left(\frac{P_X}{P_H}\right) = \pi_X \tag{7}$$

$$\log P_X = \log P_H + \pi_X \tag{8}$$

Just as Hammett had found that different σ values were required for para- and for meta-substituents on a benzoic acid, because of differing contributions of field and resonance effects on reactivity, so the Hansch group immediately recognized that different π values would be required according to the environment of the substituent. Electronic effects in particular would alter the interaction of a polar substituent with the water phase: consider 4-nitrophenol, where neither the hydroxyl group nor the nitro group would behave towards water or towards octanol in like fashion to the hydroxyl or nitro group in phenol itself, or in nitrobenzene. It was rapidly appreciated that the lipophilicity parameter, log$P$, was only to a first approximation an additive property: it has considerable constitutive character. This at first proved to be a major difficulty for the calculation of lipophilicity, but in fact opened the way to using lipophilicity measurements to probe a variety of intramolecular effects, including not only electronic but steric effects, so-called proximity effects when polar groups share a solvation shell, hydrogen bonding, and conformation (sometimes called folding effects, see chapter 4).

2.3.2 Fragment Additivity Method

The π-system was used for some 15 years, but was destined to give way to a much more general fragmentation method of calculating log*P*. The substituent scheme was only applicable, in general, to substituted benzene derivatives. For other compounds, the problem immediately arose, what does one take as "parent" and what as substituent? Moreover, rather serious errors occurred in the application and interpretation of lipophilicity calculations using the substituent approach. Hansch and Anderson [23] in 1967 suggested that the difference in calculated log*P* and in measured log*P* (which was lower) in compounds of the type $C_6H_5CH_2CH_2CH_2X$ indicated a folding of the alkyl chain, so that substituent X interacted with the aromatic ring through "intramolecular hydrophobic bonding". In 1973 Nys and Rekker [24] suggested that the difference did not arise from any intramolecular folding, but in fact arose because of the implicit neglect of the lipophilicity of hydrogen. The application of Eq. (7) to calculate log*P* for the compounds above requires the addition:

$$\log P(C_6H_5 - CH_2 - CH_2 - CH_2 - X) = \log P(C_6H_6) + 3\pi(CH_3) + \pi(X)$$

and makes no distinction between the lipophilicity of CH_3 or CH_2.

Nys and Rekker [24, 25] then suggested a totally different approach to log*P* calculation, which was to transform our understanding. This approach was based on the assignment of "fragmental constants", *f*, to a selection of structural fragments, the calculated log*P* then being simply the sum of fragment values appropriate to the molecule plus a number of interaction factors, *F*, that were necessary to correct for intramolecular electronic or steric interactions between fragments. The fragment system is expressed by Eq. (9):

$$\log P = \sum_{i=1}^{m} f_i + \sum_{i=1}^{n} F_i \tag{9}$$

Rekker used a large database of published log*P* values to derive both fragment values and correction factors statistically. His first book on the method was published in 1977 [26] and refinements were later made by Rekker and de Kort in 1979 [27] using a database of over one thousand log*P* measurements. A second book in 1992 by Rekker and Mannhold [28] includes further refinements and example calculations.

A feature of Rekker-type calculations as currently implemented is that many of the correction factors, *F*, are considered to be multiples of a so-called "magic constant", C_M, the latest value for which is 0.219 [28]. The calculation of lipophilicity therefore follows Eq. (10) with, for example, a proximity correction of *kn* (key number) equal to 2 for a two-carbon separation of polar groups:

$$\log P = \sum_{i=1}^{m} f_i + \sum kn.C_M \tag{10}$$

There has been much speculation as to whether the "magic constant" has any fundamental significance, Rekker having proposed that it might be related to a quantum displacement of water in the first solvation shell around the solute.