



Essentials of Pharmaceutical Preformulation

Simon Gaisford Mark Saunders

 **WILEY-BLACKWELL**



Rounded



Angular



Elongated

Essentials of Pharmaceutical Preformulation

Companion website

This book is accompanied by a companion website at:

<http://www.wiley.com/go/gaisford/essentials>

Visit the website for:

- Figures and tables from the book
- Multiple choice questions

Essentials of Pharmaceutical Preformulation

Simon Gaisford

UCL School of Pharmacy, University College London, London, UK

Mark Saunders

Kuecept Ltd, Potters Bar, Herts, UK



WILEY-BLACKWELL

A John Wiley & Sons, Ltd., Publication

This edition first published 2013

© 2013 John Wiley & Sons, Ltd

Registered office

John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ,
United Kingdom

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com.

The right of the author to be identified as the author of this work has been asserted in accordance with the Copyright, Designs and Patents Act 1988.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior permission of the publisher.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book. This publication is designed to provide accurate and authoritative information in regard to the subject matter covered. It is sold on the understanding that the publisher is not engaged in rendering professional services. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

Library of Congress Cataloging-in-Publication Data

Gaisford, Simon.

Essentials of pharmaceutical preformulation / Simon Gaisford and Mark Saunders.

p. ; cm.

Includes bibliographical references and index.

ISBN 978-0-470-97635-7 (cloth) – ISBN 978-0-470-97636-4 (paper)

I. Saunders, Mark, 1976 Sept. 18– II. Title.

[DNLM: 1. Drug Compounding–methods. 2. Drug Discovery–methods. QV 779]

615.1'9–dc23

2012027538

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Cover design: Gary Thompson

Set in 10.5/13pt Times Ten by Aptara Inc., New Delhi, India

First Impression 2013

For Yasmina and Oliver

Contents

Preface	xiii
List of Abbreviations	xv
1 Basic Principles of Preformulation Studies	1
1.1 Introduction	1
1.2 Assay design	2
1.2.1 Assay development	3
1.3 Concentrations	5
1.3.1 Units of concentration	5
1.4 UV spectrophotometry	9
1.4.1 Method development for UV assays	11
1.5 Thin-layer chromatography (TLC)	14
1.5.1 TLC method development	15
1.5.2 High-performance TLC	17
1.6 High-performance liquid chromatography	19
1.6.1 Normal- and reverse-phase HPLC	20
1.6.2 HPLC method development	21
1.7 Differential scanning calorimetry	22
1.7.1 Interpreting DSC data	23
1.7.2 Modulated-temperature DSC	27
1.7.3 DSC method development	30
1.8 Dynamic vapour sorption	32
1.8.1 DVS method development	32
1.9 Summary	33
References	33
Answer to study question	34
Additional study questions	35
2 Ionisation Constants	36
2.1 Introduction	36
2.2 Ionisation	36
2.2.1 Percent ionisation	42

2.3	Buffers	44
2.4	Determination of pK_a	44
2.4.1	Determination of pK_a by potentiometric titration	45
2.4.2	Determination of pK_a in nonaqueous solvents	45
2.4.3	Other factors affecting measurement of pK_a	47
2.5	Summary	48
	References	48
	Answers to study questions	49
	Additional self-study questions and answers	50
3	Partition Affinity	52
3.1	Introduction	52
3.2	Partitioning	52
3.2.1	Effect of partitioning	54
3.2.2	Determination of $\log P$	55
3.2.3	Effect of salt formation on partitioning	62
3.3	Summary	63
	References	63
	Answers to study questions	64
4	Solubility	65
4.1	Introduction	65
4.2	Intrinsic solubility	67
4.2.1	Ideal solubility	69
4.2.2	Solubility as a function of temperature	73
4.2.3	Solubility and physical form	74
4.2.4	Measurement of intrinsic solubility	77
4.2.5	Calculation of pK_a from solubility data	83
4.3	Summary	83
	References	84
	Answer to study question	84
	Additional self-study questions and answers	84
5	Dissolution	86
5.1	Introduction	86
5.2	Models of dissolution	86
5.3	Dissolution testing	87
5.3.1	Intrinsic dissolution rate (IDR)	92
5.3.2	IDR as a function of pH	93
5.3.3	IDR and the common ion effect	94
5.4	Summary	96
	References	96
	Answers to study questions	97

6	Salt Selection	98
6.1	Introduction	98
6.2	Salt formation	99
6.2.1	Selection of a salt-forming acid or base	104
6.2.2	Salt screening	108
6.3	Salt solubility	110
6.3.1	Solubility of basic salts	111
6.3.2	Solubility of acidic salts	112
6.3.3	The importance of pH_{max}	114
6.4	Dissolution of salts	117
6.4.1	Modification of pH_m	120
6.5	Partitioning of salts	121
6.6	Summary	123
	References	124
	Answers to study questions	126
7	Physical Form I – Crystalline Materials	127
7.1	Introduction	127
7.2	Crystal formation	127
7.2.1	Crystal formation from the melt	128
7.2.2	Crystal growth from solution	129
7.3	Crystal structure	130
7.4	Polymorphism	131
7.4.1	Thermodynamics of polymorphism	133
7.4.2	Physicochemical properties of polymorphs	137
7.5	Pseudopolymorphism	139
7.6	Polymorph screening	141
7.7	Characterisation of physical form	141
7.7.1	Characterisation of polymorphs	142
7.7.2	Characterisation of pseudopolymorphs	149
7.8	Summary	152
	References	152
	Answers to study questions	153
8	Physical Form II – Amorphous Materials	156
8.1	Introduction	156
8.2	Formation of amorphous materials	156
8.3	Ageing of amorphous materials	160
8.4	Characterisation of amorphous materials	162
8.4.1	Measurement of ageing	164
8.5	Processing and formation of amorphous material	168
8.5.1	Spray-drying	168
8.5.2	Freeze-drying	168
8.5.3	Quench-cooling	169

8.5.4	Milling	170
8.5.5	Compaction	171
8.6	Amorphous content quantification	171
8.6.1	Calibration standards	172
8.6.2	DSC for amorphous content quantification	173
8.6.3	DVS for amorphous content quantification	175
8.7	Summary	177
	References	178
	Answers to study questions	179
9	Stability Assessment	181
9.1	Introduction	181
9.2	Degradation mechanisms	183
9.2.1	Hydrolysis	185
9.2.2	Solvolysis	187
9.2.3	Oxidation	188
9.2.4	Photolysis	190
9.3	Reaction kinetics	191
9.3.1	Solution-phase kinetics	191
9.3.2	Zero-order reactions	192
9.3.3	First-order kinetics	193
9.3.4	Second-order reactions	194
9.3.5	Solid-state kinetics	195
9.4	The temperature dependence of reaction kinetics	198
9.5	Stress testing	203
9.5.1	Stress testing in solution	203
9.5.2	Stress testing in the solid-state	204
9.5.3	Drug–excipient compatibility testing	205
9.6	Summary	208
	References	208
	Answers to study questions	209
10	Particle Properties	211
10.1	Introduction	211
10.2	Microscopy	211
10.2.1	Light microscopy	212
10.2.2	Hot-stage microscopy	213
10.2.3	Electron microscopy	214
10.2.4	Atomic force microscopy	214
10.3	Particle shape	215
10.3.1	Habit	215
10.3.2	Particle sizing	219
10.3.3	Particle size distributions	222
10.4	Summary	227
	References	227
	Answer to study question	227

11 Powder Properties	228
11.1 Introduction	228
11.2 Powder flow and consolidation	228
11.2.1 Carr's index	230
11.2.2 Hausner ratio	232
11.2.3 Angle of repose	232
11.2.4 Mohr diagrams	235
11.3 Compaction properties	240
11.3.1 Compaction simulators	242
11.4 Summary	243
References	243
Answers to study questions	243
Index	247

Companion website

This book is accompanied by a companion website at:

<http://www.wiley.com/go/gaisford/essentials>

Visit the website for:

- Figures and tables from the book
- Multiple choice questions

Preface

The inspiration for this book came when Michael Aulton asked me to take over his preformulation module on the PIAT course of the University of Manchester. The existing module was based on the excellent textbook (*Pharmaceutical Preformulation*) written by Jim Wells in 1988 and thus a perfect opportunity to write both an updated module and an updated book presented itself.

The majority of the text was written while I was on sabbatical leave at the Monash Institute for Pharmaceutical Sciences (MIPS) in Melbourne, Australia, in the summer of 2011. I am hugely grateful to Prof. Bill Charman, Prof. Peter Stewart, Marian Costelloe and Marian Glennon for arranging the visit and to MIPS as a whole for the welcoming, friendly and stimulating environment they provided. I would also like to mention many of the wonderful people that I met, including Anne, Ben, Carolyn, Chris, Colin, David, Ian, Hywel, Iliana, Joe, Laurence, Mercedes and Michelle. Special thanks are reserved for Richard Pranker, who took the time and effort to talk with me about many aspects of the text while I was at MIPS and also to review the whole text before publication; the book is immeasurably better for his input and advice. Thermal analysts are indeed a special breed!

Equally, the book would never have been finished were it not for several other special people. Nicole Hunter undertook the weighty tasks of reviewing the whole text and providing constant support and encouragement while Hamid Merchant cast his expert eye over the dissolution chapter. My group of amazing PhD students (Alice, Asma, Garima, Jawal, Jip, Luis, Mansa, Mustafa and Rin) provided many of the data and examples that populate the text while my academic colleagues, particularly Prof. Abdul Basit, Prof. Anthony Beezer and Prof. Kevin Taylor, have been constant sources of advice and support. I also acknowledge all of the wonderful students who I have taught on the MSc in Drug Delivery and who are such an inspiration to me.

Of course, no book would be possible without a publisher, and I am extremely grateful to Fiona Seymour and Lucy Sayer for editorial advice and encouragement.

Finally, I must acknowledge the constant support of my family, especially Joanne and Oliver, who keep me sane!

S Gaisford

April 2012

List of Abbreviations

Abbreviation	Meaning
ε	Molar absorption coefficient
θ	Angle of repose
σ	Normal stress
τ	Shear stress
A	Surface area
AFM	Atomic force microscopy
AR	Aspect ratio
BCS	Biopharmaceutical Classification System
BP	British Pharmacopoeia
C	Concentration
CRM	Certified reference material
D	Diffusion coefficient
DSC	Differential scanning calorimetry
ESEM	Environmental scanning electron microscopy
F	Dilution factor
FaSSIF	Fasted state simulated intestinal fluid
FDA	US Food and Drug Administration
FeSSGF	Fed state simulated gastric fluid
FeSSIF	Fed state simulated intestinal fluid
FTIR	Fourier transform infrared
G	Gibb's free energy
GRAS	Generally regarded as safe
h	Thickness of boundary layer
H	Enthalpy
HPLC	High-performance liquid chromatography
HSM	Hot-stage microscopy
ICH	International Conference on Harmonisation

IDR	Intrinsic dissolution rate
IR	Infrared
IUPAC	International Union of Pure and Applied Chemistry
<i>k</i>	Rate constant
<i>K</i>	Equilibrium constant or stress ratio
MTDSC	Modulated temperature DSC
<i>n</i>	Reaction order
NA	Numerical aperture
NIR	Near infrared
<i>p</i>	Negative logarithm
PhEur	European Pharmacopoeia
RI	Refractive index
<i>S</i>	Entropy
<i>S</i> ₀	Intrinsic solubility
SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
<i>T</i>	Temperature
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
TLC	Thin-layer chromatography
UHPLC	Ultra high-performance liquid chromatography
USP	United States Pharmacopoeia
UV	Ultraviolet
<i>V</i>	Volume
<i>W</i>	Weight
<i>x</i>	Mole fraction
XRPD	X-ray powder diffraction

1

Basic Principles of Preformulation Studies

1.1 Introduction

The worldwide market for pharmaceutical sales is large and has grown consistently year-on-year for much of the past decade (Table 1.1). The advent of computer-based drug design programmes, combinatorial chemistry techniques and compound libraries populated with molecules synthesised over many decades of research and development means there is a vast array of compounds with the potential to become drug substances. However, drug substances are not administered to patients as pure compounds; they are formulated into drug products. The selection of a compound, its development into a drug substance and, ultimately, drug product is a hugely time-consuming and expensive process, which is ultimately destined for failure in the majority of cases. As a rough guide, only 1 out of every 5–10 000 promising compounds will be successfully developed into a marketed drug product and the costs involved have been estimated at ca. \$1.8 billion (Paul *et al.*, 2010).

While it is tempting to assume that all drug products are financial blockbusters, approximately 70% never generate sufficient sales to recoup their development costs. Table 1.2 shows the top 20 medicines by sales worldwide (and the percentage of revenue they generate for their respective companies). It is apparent that a significant percentage of income is generated from these blockbuster products, and the financial health and prospects of the originator company are largely dependent upon the extent of patent protection (allowing market exclusivity) and new drug products in the development pipeline.

These numbers imply that development of a drug product in the right therapeutic area can result in significant income, but the costs involved in

Table 1.1 Total market sales in the pharmaceutical sector from 2003 to 2010 (data from IMS Health).

	2003	2004	2005	2006	2007	2008	2009	2010
Total market ^a	500	560	605	651	720	788	819	856
% Growth	9.1	7.6	7.2	7.0	6.9	6.1	7.1	4.1

^aUS\$ in billions.

reaching market are such that only a few potential drug substances can be considered for development. How best to select a compound for development from the myriad of chemical structures that may be available? It is tempting to think that the decision reduces to efficacy against a biological target alone, but in practice physicochemical properties affect how a substance will process, its stability and interaction with excipients, how it will transfer to solution and, ultimately, define its bioavailability. The compound showing greatest efficacy may not ultimately be selected if another compound has a better set of physicochemical properties that make it easier to formulate and/or manufacture. It follows that characterising the physicochemical properties of drug substances early in the development process will provide the fundamental knowledge base upon which candidate selection, and in the limit dosage form design, can be made, reducing development time and cost. This is the concept of preformulation.

1.2 Assay design

In the early stages of preformulation the need rapidly to determine bioavailability, dose and toxicity data predominate and hence the first formulations

Table 1.2 Top ten drugs by sales worldwide in 2010 (data from IMS Health).

Product	Manufacturer	Sales (US\$m)	% of company sales	Date of patent expiry
Lipitor	Pfizer	12 657	22.8	2011
Plavix	Sanofi-Aventis/BMS	8817	17.3 ^a	2012
Seretide	GlaxoSmithKline	8469	25.2	2013 ^b
Nexium	AstraZeneca	8362	23.5	2014
Seroquel	AstraZeneca	6816	19.2	2012
Crestor	AstraZeneca	6797	19.1	2012
Enbrel	Amgen/Pfizer	6167	8.7 ^a	2012
Remicade	Janssen/Schering-Plough	6039	n/d	2011
Humira	Abbott	5960	25.0	2016
Zyprexa	Eli Lilly	5737	25.9	2011

^aBased on combined sales of both companies.^bEuropean expiry. The US patent expired for Seretide in 2010.

Table 1.3 Molecular sample properties and the assays used to determine them.

Property	Assay	Requirement of sample
Solubility ^a	UV	Chromophore
<ul style="list-style-type: none"> • Aqueous • Nonaqueous 		
pK _a	UV or potentiometric titration	Acid or basic group
P _{o, w} /log P	UV TLC HPLC	Chromophore
Hygroscopicity	DVS TGA	No particular requirement
Stability	HPLC, plus suitable storage conditions	No particular requirement
<ul style="list-style-type: none"> • Hydrolysis • Photolysis • Oxidation 		

^aSolubility will depend on physical form.

of a drug substance are usually for intravenous injection. The first task facing any formulator is thus to prepare a suitable formulation for injection – most often this requires only knowledge of solubility and the development of a suitable assay. It is extremely important to note here that no development work can proceed until there is a suitable assay in place for the drug substance. This is because experimentation requires measurement.

1.2.1 Assay development

Assays greatly assist quantitative determination of physicochemical parameters. Since each assay will in general be unique to each drug substance (or, more correctly, *analyte*) development of assays may be time-consuming in cases where many drug substances are being screened. The first assays developed should ideally require minimum amounts of sample, allow determination of multiple parameters and be applicable to a range of compounds. For instance, a saturated solution prepared to determine aqueous solubility may subsequently be used to determine partition coefficient, by addition of *n*-octanol.

Note at this stage that determination of *approximate* values is acceptable in order to make a go/no go decision in respect of a particular candidate and so assays do not need to be as rigorously validated as they do later in formulation development. Table 1.3 lists a range of molecular properties to be measured during preformulation, in chronological order, and the assays that may be used to quantify them. These properties are a function of

Table 1.4 Macroscopic (bulk) sample properties and the techniques used to determine them.

Derived property	Technique
Melting point	DSC or melting point apparatus
Enthalpy of fusion (and so ideal solubility)	DSC
Physical forms (polymorphs, pseudopolymorphs or amorphous)	DSC, XRPD, microscopy
Particle shape	Microscopy
• Size distribution	Particle sizing
• Morphology	BET (surface area)
• Rugosity	
• Habit	
Density	Tapping densitometer
• Bulk	
• Tapped	
• True	
Flow	Angle of repose
Compressibility	Carr's index Hausner ratio
Excipient compatibility	HPLC, DSC

molecular structure. Once known, further macroscopic (or bulk) properties of the drug candidate can be measured (Table 1.4). These properties result from intermolecular interactions. Note also that determination of chemical structure does not appear, as it is assumed that the chemists preparing the candidate molecules would provide this information. Note also that solubility will be dependent upon physical form (polymorph, pseudopolymorph or amorphous).

Full characterisation of a drug substance should be possible with just five techniques: ultraviolet (UV) spectrophotometry, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), differential scanning calorimetry (DSC) and dynamic vapour sorption (DVS). This explains the popularity of these techniques in pharmaceutical development laboratories and so their basic principles are outlined below. Other, more specialised techniques (such as X-ray powder diffraction, XRPD) provide additional information. Application of the techniques is discussed in later chapters, but the basic principles are discussed below.

Note that in the limit the sensitivity of the assay will be dependent upon the purity of the sample (greater levels of impurity lowering sensitivity) and so assay development should be undertaken with the purest sample obtainable. Sensitivity can be expressed in many ways, but commonly detection limits (DL) or quantification limits (QL) are specified. There are many ways of

calculating DL and QL values. ICH Guideline Q2(R1) (2005) defines the following:

$$DL = \frac{3.3\sigma}{s} \quad (1.1)$$

$$QL = \frac{10\sigma}{s} \quad (1.2)$$

where σ is the standard deviation of the blank measurement and s is the slope of the calibration plot. Since all assays require understanding of concentration terms, these concepts will be discussed first.

1.3 Concentrations

Concentration terms simply define the ratio of two components in a particular sample. The minor component is termed the solute and the major component is termed the solvent. It does not matter what the physical forms of the solute and solvent are (i.e. they can be solid, liquid or gas, although certain combinations are not usually encountered, such as a gas dissolved in a solid).

Importantly, a concentration term specifies the amount of solute present *per unit of solvent*. Thus, defining a concentration gives no information on how large the sample is; everything is normalised to a particular unit. So, for instance, if a sample is defined as a 1 M aqueous solution of aspirin, there is a mole of aspirin in every litre of water. It is not possible to know from this statement how much solution there is. If, instead, the sample was defined as 500 mL of a 1 M aqueous solution of aspirin, there is sufficient information to know everything about what material is present and in what quantity.

1.3.1 Units of concentration

The amounts of solute and solvent can be specified a number of ways. The most commonly encountered units in pharmaceuticals are:

- Molar (M, moles per litre)
- Molal (m, moles per kg)
- Percentages (w/w, w/v, v/v)
- Weight per volume (mg mL^{-1})
- Parts per million (ppm)
- Mole fraction (x)

Since it is possible to define concentrations with a multitude of terms, care must be taken when comparing systems expressed in different units. The major issue to be accounted for is the molecular mass of the solute.

Example 1.1 Which of the following pairs of solutions (assume there is 1 L of each) contains the same number of solute molecules?

- (a) 2 % w/v formoterol fumarate and 2 % w/v salbutamol sulphate
- (b) 0.1 M formoterol fumarate and 0.1 M salbutamol sulphate

The answer is (b), because the amount of solute is expressed in terms of molarity, which is independent of molecular weight.

For small organic molecules, such as the majority of drugs, differences in the number of molecules between solutions expressed in weight percentages may be small, but as the molecular weight of the solute increases (where polymeric excipients are used, for instance) the differences can become significant. Care must be taken when constructing and interpreting an experimental series based on percentage concentrations that differences observed between solutes do not arise simply as a result of different numbers of solute molecules per unit volume.

Molar concentrations avoid this problem and so molar is the standard unit of concentration used in the SI¹ (le Système International d'Unités) nomenclature. If Z is the molecular weight of a solute, then Z grams of that solute contains 1 mole (6.022×10^{23}) of molecules.

The difference between molar (M) and molal (m) is the same as the difference between % w/v and % w/w (i.e. 1 M is 1 mole per litre while 1 m is 1 mole per kilogram).

In pharmaceuticals the molarities of typical solutions may be very low and hence the most frequently encountered units are those based on weight or volume fractions. Many dosage forms are solids and thus are more amenable to percentage concentration expressions. Also, if the molecular weight of a new drug substance is not known, then it is not possible to calculate molar or molal concentrations.

Example 1.2 What do the following concentration terms mean?

- (a) 0.1% w/v
- (b) 2% w/w

¹Interestingly, three countries have not adopted SI nomenclature, Liberia, Burma and the United States, although as of 2010 Liberia is gradually introducing metric units. The United Kingdom uses an eclectic mix of SI units in science and metric and Imperial units in everyday life.

In the case of (a) the concentration term (w/v) implies a solid solute has been dissolved in a liquid solvent; 0.1% implies that the ratio of solute to solvent is 0.1:100. So 0.1% w/v means 0.1 g of solute in 100 mL of solvent.

In the case of (b) the concentration term (w/w) implies a solid solute has been dissolved in a solid solvent; 2% implies that the ratio of solute to solvent is 2:100. So 2% w/w means 2 g of solute in 100 g of solvent.

Another point to remember is that percentage terms are expressed per 100 mL of solvent while molar terms are expressed per litre of solvent. Although weight percentage terms are common in pharmaceuticals, again the low concentrations often used make the numbers small. Also, many medicines are defined as weight of drug per unit dose (50 mg per tablet for instance), so weights per unit volume concentrations are very often used:

- 2 mg mL⁻¹
- 50 mg L⁻¹
- 10 g L⁻¹

Example 1.3 Do the following solutions contain equal numbers of molecules?

- (a) 5 mg mL⁻¹ paracetamol and 5 mg mL⁻¹ ibuprofen
- (b) 10 mg mL⁻¹ nicotinamide and 10 mg mL⁻¹ isonicotinamide

Not in the case of (a) as the molecular weights of the drug substances are different. The only concentration terms that normalise for numbers of molecules are molarity or molality. In the special case (b) the drug substances have the same molecular weight and so the numbers of molecules are equal.

The term ppm is less commonly encountered in pharmaceuticals, being more associated with gases or very dilute contaminants in solution; 1 ppm means 1 part of solute to a million parts of solvent (easily remembered as 1 mg per litre).

There is one further way of expressing concentration: mole fraction (x). The mole fraction of a component is defined as the number of moles of that component divided by the total number of moles of all of the components in the system:

$$x_a = \frac{\text{Number of moles of component } a}{\text{Total number of moles of all components in system}} \quad (1.3)$$

Mole fractions are dimensionless and must always have a value between 0 and 1. The sum of the mole fractions of all the components in a system must

equal 1. Mole fraction units are useful if there are two or more solutes in the same solvent.

Example 1.4 A solution for intravenous injection is prepared at 25 °C with the following constituents: water (50 g, RMM 18), lidocaine hydrochloride (1 g, RMM 270.8) and epinephrine (0.5 mg, RMM 183.2). Calculate:

- The mole fraction of lidocaine hydrochloride
- The mole fraction of epinephrine
- The mole fraction of water

Firstly, the number of moles of each component must be calculated:

$$\text{Number of moles of lidocaine hydrochloride} = \frac{1}{270.8} = 0.00369$$

$$\text{Number of moles of epinephrine} = \frac{0.0005}{183.2} = 0.00000273$$

$$\text{Number of moles of water} = \frac{50}{18} = 2.78$$

and so

$$x_{\text{lidocaine HCL}} = \frac{0.00369}{0.00369 + 0.00000273 + 2.78} = 0.00133$$

$$x_{\text{epinephrine}} = \frac{0.00000273}{0.00369 + 0.00000273 + 2.78} = 0.00000098$$

$$x_{\text{water}} = 1 - 0.00133 - 0.00000098 = 0.9987$$

Summary box 1.1

- Concentrations define the amount of solute per unit volume or mass of solvent.
- Molar or molal concentrations can be compared in terms of numbers of solute molecules.
- Percent or weight/volume terms are more common in pharmaceuticals and can be converted to molar/molal concentrations if the molecular weight of the solute is known.
- 1% w/v $\equiv 10 \text{ g L}^{-1} \equiv 10 \text{ mg mL}^{-1}$
- 1 ppm $\equiv 1 \text{ mg L}^{-1}$

Table 1.5 UV absorbance maxima for a range of common functional groups (data from Wells (1988)).

Chromophore	λ_{\max} (nm)	Molar absorption (ϵ)
Benzene	184	46 700
Naphthalene	220	112 000
Anthracene	252	199 000
Pyridine	174	80 000
Quinoline	227	37 000
Ethylene	190	8000
Acetylide	175–180	6000
Ketone	195	1000
Thioketone	205	Strong
Nitrite	160	–
Nitroso	302	100
Nitro	210	Strong
Amino	195	2800
Thiol	195	1400
Halide	208	300

1.4 UV spectrophotometry

Unless there is a good reason not to, the primary assay developed during preformulation will be based on UV spectrophotometry. Many factors contribute to the popularity of the technique, including familiarity, cost, amount of solution used and the fact that the majority of drug substances contain at least one functional group that absorbs in the ultraviolet (UV) region (190–390 nm). Table 1.5 lists the UV absorbance maxima for a series of common functional groups (called *chromophores*).

Since a chromophore is a functional group with absorption in the UV range, excitation of the solute with the appropriate wavelength of light will reduce the amount of light passing through the solution. If the original light intensity is I_0 and the amount of light passing through the sample (the *transmitted light*) is I , then the amount of light absorbed will be a function of the concentration of the solute (C) and the depth of the solution through which the light is passing (the path length, l), usually expressed as the Beer–Lambert equation:

$$\text{Absorbance} = \log \frac{I_0}{I} = \epsilon Cl \quad (1.4)$$

where ϵ is a constant of proportionality called the molar absorption coefficient. Higher values of ϵ mean greater absorbance by the solute. Values of ϵ for a range of functional groups are given in Table 1.5; it can be seen that groups containing large numbers of delocalised electrons, such as those