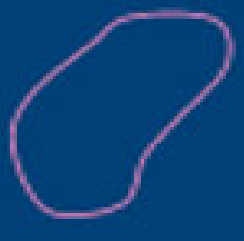
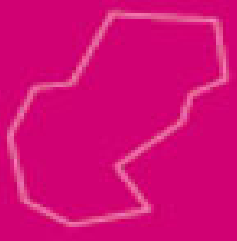
Essentials of Pharmaceutical Preformulation

Simon Gaisford Mark Saunders

WILEY-BLACKWELL





Contents

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

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

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

First Impression 2013

For Yasmina and Oliver

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 Prankerd, 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
k	Rate constant
K	Equilibrium constant or stress ratio
MTDSC	Modulated temperature DSC
n	Reaction order
NA	Numerical aperture
NIR	Near infrared
p	Negative logarithm
PhEur	European Pharmacopoeia
RI	Refractive index
S	Entropy
S_o	Intrinsic solubility
SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
T	Temperature
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
TLC	Thin-layer chromatography
UHPLC	Ultra high-performance liquid chromatography
USP	United States Pharmacopoeia
UV	Ultraviolet
V	Volume
W	Weight
x	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).

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

[a]US$ in billions.

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.

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

[a]Based on combined sales of both companies.
[b]European expiry. The US patent expired for Seretide in 2010.

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

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

Property	Assay	Requirement of sample
Solubility[a] • Aqueous • Nonaqueous	UV	Chromophore
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 • Hydrolysis • Photolysis • Oxidation	HPLC, plus suitable storage conditions	No particular requirement
[a]Solubility will depend on physical form.		

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 • Size distribution • Morphology • Rugosity • Habit	MicroscopyParticle sizingBET (surface area)
Density • Bulk • Tapped • True	Tapping densitometer
Flow	Angle of repose
Compressibility	Carr's indexHausner ratio
Excipient compatibility	HPLC, DSC

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, XPRD) 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:

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

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

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

pharmaceutics 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 SI[1] (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 pharmaceutics 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

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 pharmaceutics, 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} nicatinamide and 10 mg mL^{-1} isonicatinamide

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 pharmaceutics, 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 pharmaceutics and can be converted to molar/molal concentrations if the molecular weight of the solute is known.
- $1\%\ \text{w/v} \equiv 10\ \text{g L}^{-1} \equiv 10\ \text{mg mL}^{-1}$
- $1\ \text{ppm} \equiv 1\ \text{mg L}^{-1}$

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*).

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
Ethlyene	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

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:

$$\text{Absorbance} = \log \frac{I}{I_0} = \varepsilon C l \quad (1.4)$$

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 containing benzene rings, have much greater ε values than groups containing simple carbon–carbon double bonds. Many drug substances contain aromatic moieties of carbon–carbon double bonds, which is why UV spectroscopy is a good first choice assay.

The absorbance of a chromophore can be affected by the presence of an adjacent functional group if that group has unshared electrons (an *auxochrome*). A list of common auxochromes and their effects on the molar absorption coefficients of their parent benzene ring is given in Table 1.6.

Table 1.6 The effect of auxochromes on the UV absorbance of the parent compound C_6H_5–R (data from Wells (1988)).

Substituent	λ_{max} (nm)	Molar absorption (ε)
–H	203.5	7400
$-CH_3$	206.5	7000
–Cl	209.5	7400
–OH	210.5	6200
$-OCH_3$	217	6400
–CN	224	13 000
$-COO^-$	224	8700
$-CO_2H$	230	11 600
$-NH_2$	230	8600
$-NHCOCH_3$	238	10 500
$-COCH_3$	245.5	9800
$-NO_2$	268.5	7800

Use of the molar absorption coefficient is fine when concentrations are expressed in molar terms. However, as noted above, in pharmaceutics it is more common to express concentrations in percentage terms, in which case an alternative constant of proportionality must be defined. This is usually of the form of a specific absorption coefficient (A). Although any such constant may be defined, usually the

reference value is the absorbance of a 1% w/v solution in a 1 cm path length UV cuvette:

$$\text{Specific absorption coefficient} = A^{1\%}_{1\text{ cm}} \tag{1.5}$$

The Beer–Lambert equation therefore becomes

$$\text{Absorbance} = A = A^{1\%}_{1\text{ cm}} Cl \tag{1.6}$$

Assuming a 1 cm path length cuvette is used then

$$C = \frac{A}{A^{1\%}_{1\text{ cm}}} \tag{1.7}$$

Knowledge of the value of $A^{1\%}_{1\text{ cm}}$ allows determination of the concentration of a solution by measurement of its absorbance (and will yield an answer in % w/v). Values of $A^{1\%}_{1\text{ cm}}$ are often quoted in pharmacopoeial monographs.

Example 1.5 What is the concentration of a solution of buflomedil hydrochloride that gives an absorbance reading of 0.5 at 275 nm ($A^{1\%}_{1\text{ cm}}$ = 143 at 275 nm)?

From Equation (1.5),

$$C = \frac{0.5}{143} = 0.0035\%\text{w/v}$$

As might be expected, the molar absorption coefficient and the specific absorption coefficient are related through the molecular mass of the solute.

Study question 1.1 Show that the molar absorption coefficient (ε) and the specific absorption coefficient ($A^{1\%}_{1\text{ cm}}$) are related according to the following relationship:

$$\varepsilon = A^{1\%}_{1\text{ cm}} \times \left(\frac{\text{Molecular weight}}{10}\right)$$

The $A^{1\%}_{1\text{ cm}}$ value for most drug substances lies in the range 200–1000 with a mean of ca. 500. A 10 μg mL^{-1} solution of a drug with $A^{1\%}_{1\text{ cm}}$ = 500 would give an absorbance of 0.5, well within the range of UV spectrophotometers. Compounds with $A^{1\%}_{1\text{ cm}}$ < 50 are generally too poorly absorbing for successful UV analysis.

1.4.1 Method development for UV assays

If the compound has good aqueous solubility then water is the most appropriate solvent. Frequently, however, a drug substance will have poor aqueous solubility, in which case an alternative strategy is required. Methanol is a good solvent as a first attempt, as it is a good solvent for both polar and nonpolar drugs, it does not have significant UV absorbance and solubility is often nearer to ideal (see Chapter 4). An additional benefit is that it is miscible with water, so the drug substance can initially be dissolved in a small volume of methanol and then diluted with water. Dilution is best achieved with 0.1 M HCl or NaOH (as appropriate, depending upon whether the drug substance is a weak acid or base) since this will maximise ionisation, and hence solubility, and neither solute absorbs in the UV region.

Other solvents may be used in UV spectroscopy but their UV cut-off (the wavelength below which they absorb significantly) may differ from water (Table 1.7). In the worst case a solubilising agent can be added, but care must be taken to ensure it does not absorb in the UV range or, if it does, to correct for the absorbance with a suitable blank.

Table 1.7 Suitable solvents for UV analysis.

Solvent	UV cut-off wavelength (nm)
Water	200
Methanol	200
Ethanol (96%)	200
Propanol	200
Isopropanol	210
Butanol	210
Pentanol	210
n-Hexane	210
n-Heptane	210
Glycerol	200
Acetonitrile	200
Cyclohexane	210

Solvent	UV cut-off wavelength (nm)
iso-Octane	210
Dioxane	220
Ethyl ether	220
Chloroform	245

The performance of the instrument should be checked prior to use with reference standards. (This is true for all analytical instruments. Institutions such as the National Institute for Standards and Technology, NIST, or the Laboratory of the Government Chemist, LGC, can advise on and supply certified reference materials, CRMs). A solution of holmium oxide in perchloric acid solution can be used for wavelength calibration (Weidner *et al.*, 1985) and is specified in the Ph Eur, while a solution of potassium dichromate can be used to check absorbance (Burke and Mavrodineanu, 1976, 1977). The properties of these solutions, as well as typical specifications for a well-performing instrument are given in Table 1.8.

Table 1.8 Specifications for a UV spectrometer (data from Wells (1988)).

Attribute	Specification		
Wavelength (λ)	Holmium oxide in perchloric acid solution ±1 nm between 200 and 400 nm		
Characteristic maxima	241.15, 287.15, 361.50 and 536.30 nm		
Absorbance	Potassium dichromate solution		
	λ	$A^{1\%}_{1\,cm}$	$A^{1\%}_{1\,cm}$ limit
	235 nm	124.5	±1.7
	257 nm	144	±1.7
	313 nm	48.6	±1.7
	350 nm	106.6	±1.7
Cuvettes	Quartz, path length ±0.005 cm		
Solvents	Absorbance <0.4 (ideally <0.2) relative to air		
Path length	Usually 1 cm, although 1 mm cuvettes available		
Temperature	20 ± 1 °C		

A full spectrum scan of the solution will allow assessment of the most appropriate wavelength (λ) for analysis. Ideally, this would be the wavelength at which maximum absorption is seen (λ_{max}), corresponding to the tip of an absorbance