Pharmaceutics

The Science of Dosage Form Design

Edited by

Michael E. Aulton BPharm PhD FAAPS MRPharmS
Professor of Pharmaceutical Technology,
School of Pharmacy,
De Montfort University,
Leicester, UK

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Contents

8

Pharmaceutical preformulation: the physicochemical properties of drug substances

James Wells

CHAPTER CONTENTS

THE CONCEPT OF PREFORMULATION

Almost all new drugs are marketed as tablets, capsules or both (Table 8.1). Although only a few are marketed as an injection (25% of those marketed as tablets) the intravenous route is always required during early toxicity, metabolic, bioavailability and clinical studies to provide a precise drug and dose deposition. Other dosage forms may be required (Table 8.1) but these are drug specific and depend to a large extent on the successful development of tablets, capsules and injections.

Prior to the development of these three major dosage forms, it is essential that certain ***fundamental*** physical and chemical properties of the drug molecule and other ***derived*** properties of the drug powder are determined. This information dictates many of the subsequent events and approaches in formulation development. This first learning phase is known as ***preformulation***.

A recommended list of the information required in preformulation is shown in Table 8.2. This is assembled, recognizing the relative importance and probable existence of only limited quantities of new bulk drug (mg rather than g). Investigators must be pragmatic and generate data of immediate relevance, especially if the likely dosage forms are known.

Two fundamental properties are mandatory for a new compound:

1. Intrinsic solubility (C_o),
2. Dissociation constant (pK_a).

Table 8.1 Frequency distribution of dosage form types manufactured in the UK

Dosage form	Frequency (%)
Tablets	46
Liquid oral	16
Capsules	15
Injections	13
Suppositories and pessaries	3
Topicals	3
Eye preparations	2
Aerosols (inhalation)	1
Others	1

Independent of this pharmaceutical profiling (Table 8.2), analysts will generate data (Table 8.3) to confirm structure and purity, and this should be used to complement and confirm pharmaceutical data. Their greater training and knowledge in analysis will assist in the identification of suitable stability-indicating assays by high-performance liquid chromatography (HPLC).

SPECTROSCOPY

The first step in preformulation is to establish a simple analytical method. Most drugs absorb light in the ultraviolet wavelengths (190–390 nm) as they are

Table 8.2 Preformulation drug characterization

Test	Method/function/characterization
Spectroscopy	Simple UV assay
Solubility	Phase solubility, purity
aqueous	Intrinsic solubility, pH effects
pK_a	Solubility control, salt formation
salts	Solubility, hygroscopicity, stability
solvents	Vehicles, extraction
partition coeff K^o_w	Lipophilicity, structure activity
dissolution	Biopharmacy
Melting point	DSC – polymorphism, hydrates, solvates
Assay development	UV, TLC, HPLC
Stability (in solution and solid state)	Thermal, hydrolysis, oxidation, photolysis, metal ions, pH.
Microscopy	Morphology, particle size
Powder flow	Tablet and capsule formulation
bulk density	
angle of repose	
Compression properties	Tablet and capsule formation
Excipient compatibility	Excipient choice

Table 8.3 Analytical preformulation

Attribute	Test
Identity	Nuclear magnetic resonance (NMR) Infra red spectroscopy (IR) Ultraviolet spectroscopy (UV) Thin-layer chromatography (TLC) Differential scanning calorimetry (DSC) Optical rotation, where applicable
Purity	Moisture (water and solvents) Inorganic elements Heavy metals Organic impurities Differential scanning calorimetry (DSC)
Assay	Titration Ultraviolet spectroscopy (UV) High-performance liquid chromatography (HPLC)
Quality	Appearance Odour Solution colour pH of slurry (saturated solution) Melting point

generally aromatic and contain double bonds. The acidic or basic nature of the molecule can be predicted from functional groups (Perrin et al 1981). Using the UV spectrum of the drug, it is possible to choose an analytical wavelength (often λ_{max}) suitable to quantify the amount of drug in a particular solution. Excitation of the molecule in solution causes a loss in light energy, and the net change from the intensity of the incident light (I_o) and the transmitted light (I) can be measured. The amount of light absorbed by a solution of drug is proportional to the concentration (C) and the path length of the solution (l) through which the light has passed. Equation 8.1 is the Beer–Lambert law, where e is the molar extinction coefficient.

$$\text{Absorbance } (A) = \log_{10} (I_o/I) = eCl \qquad (8.1)$$

In pharmacy it is usual to use the ***specific absorption coefficient*** $E_{1\,cm}^{1\%}$ (E_1^1), where the pathlength is 1 cm and the solution concentration is 1% w/v (10 mg mL^{-1}), as doses of drugs and concentrations are generally in unit weights rather than molarity ($E_1^1 = 10e/MW$).

SOLUBILITY

Aqueous solubility

The availability of a drug is always limited and the preformulation scientist may only have 50 mg. As the compound is new the quality is invariably poor, so that a large number of impurities may be present and often the first crystals come down as a metastable polymorph. Accordingly, as a minimum, the solubility and pK_a must be determined. Solubility dictates the ease with which formulations for oral gavage and intravenous injection studies in animals are obtained. The pK_a allows the informed use of pH to maintain solubility and to choose salts required to achieve good bioavailability from the solid state (Chapter 9) and improve stability (Chapter 7) and powder properties (Chapter 13 and 14).

Kaplan (1972) suggested that unless a compound has an aqueous solubility in excess of 1% (10 mg mL^{-1}) over the pH range 1–7 at 37°C, potential bioabsorption problems may occur. If the intrinsic dissolution rate was greater than 1 mg cm^{-2} min^{-1} then absorption was unimpeded. Dissolution rates less than 0.1 mg cm^{-2} min^{-1} were likely to give dissolution rate-limited absorption. This tenfold difference in dissolution rate translates to a lower limit for solubility of 1 mg mL^{-1}. Under sink conditions, dissolution rate and solubilities are proportional.

A solubility of less than 1 mg mL^{-1} indicates the need for a salt, particularly if the drug will be formulated as a tablet or capsule. In the range 1–10 mg mL^{-1} serious consideration should be given to salt formation. When the solubility of the drug cannot be manipulated in this way (neutral molecules, glycosides, steroids, alcohols, or where the pK_a is less than 3 for a base or greater than 10 for an acid) then liquid filling in soft or hard gelatin capsules may be necessary.

Intrinsic solubility (C_o)

An increase in solubility in acid compared to aqueous solubility suggests a weak base, and an increase in alkali a weak acid. In both cases a dissociation constant (pK_a) can be measured and salts should form. An increase in acidic and alkaline solubility suggests either amphoteric or zwitterion behaviour. In this case there will be two pK_as, one acidic and one basic. No change in solubility suggests a non-ionizable neutral molecule with no measurable pK_a, and solubility manipulation will require either solvents or complexation.

When the purity of the drug sample can be assured, the solubility value obtained in acid for a weak acid or alkali for a weak base can be assumed to be the intrinsic solubility (C_o), ie. the fundemental solubility when completely unionized. The solubility should ideally be measured at two temperatures:

1. 4°C to ensure physical stability and extend short-term storage and chemical stability until more definitive data are available. The maximum density of water occurs at 4°C. This leads to a minimum aqueous solubility.
2. 37°C to support biopharmaceutical evaluation.

However, as absolute purity is often in doubt it is more accurate to determine this crucial solubility by the use of a phase-solubility diagram (Fig. 8.1). The data are obtained from a series of experiments in which the ratio of the amount of drug to the amount of dissolving solvent is varied.

Any deviation from the horizontal is indicative of impurities, which a higher drug loading and its inherent impurities either promotes or suppresses solubility. In cases where the observed result changes with the amount of solvent, the line is extrapolated to zero phase ratio, where solubility will be independent of solvent level and the true intrinsic solubility of the drug. The United States Pharmacopoeia uses this method to estimate the purity of mecamylamine hydrochloride.

pK_a from solubility data

Seventy-five per cent of all drugs are weak bases; 20% are weak acids and only 5% are non-ionic, amphoteric or alcohols. It is therefore appropriate to consider the Henderson–Hasselbalch equations for weak bases and acids.

For weak bases: $pH = pK_a + \log_{10}([B])/[BH^+])$ (8.2)

and for weak acids: $pH = pK_a + \log_{10}([A^-])/[HA])$ (8.3)

Equations 8.2 and 8.3 can be used:

1. to determine pK_a by following changes in solubility
2. to predict solubility at any pH, provided that the intrinsic solubility (C_o) and pK_a are known
3. to facilitate the selection of suitable salt-forming compounds and predict the solubility and pH properties of the salts.

Albert and Serjeant (1984) give a detailed account of how to obtain precise pK_a values by potentiometry, spectroscopy and conductivity.

Salts

A major improvement in solubility can be achieved by forming a salt. Acceptable pharmaceutical salt counter-ions are shown in Table 8.4. As an example, the consequence of changing chlordiazepoxide to various salt forms is shown in Table 8.5.

In some cases, salts prepared from strong acids or bases are freely soluble but very hygroscopic. This does lead to instability in tablet or capsule formulations, as some drug will dissolve in its own adsorbed films of moisture. It is often better to use a weaker acid or base to form the salt, provided any solubility requirements are met. A less soluble salt will gener-

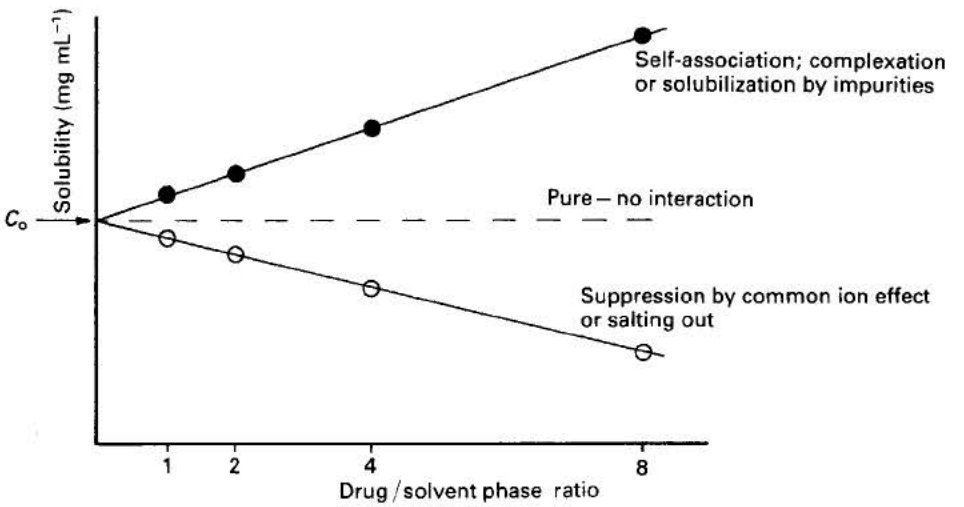

Fig. 8.1 Effect of drug: solvent ratio on solubility when the drug is impure. Assuming the compound is a base and the estimate of its solubility in 0.1 M NaOH was 1 mg mL^{-1}, four solutions of 3 mL should be set up containing 3, 6, 12 and 24 mg of drug. These give the phase ratios shown here. 3 mL is the smallest volume that can be manipulated for either centrifugation or filtration and dilution of UV analysis. The vials should be agitated continuously overnight and then the concentration in solution determined.

Table 8.4 Potential pharmaceutical salts

Basic drugs			Acidic drugs		
Anion	pK_a	% Usage	Cation	pK_a	% Usage
Hydrochloride	−6.10	43.0	Potassium	16.00	10.8
Sulphate	−3.00, +1.96	7.5	Sodium	14.77	62.0
Mesylate	−1.20	2.0	Lithium	13.82	1.6
Maleate	1.92, 6.23	3.0	Calcium	12.90	10.5
Phosphate	2.15, 7.20, 12.38	3.2	Magnesium	11.42	1.3
Salicylate	3.00	0.9	Diethanolamine	9.65	1.0
Tartrate	3.00	3.5	Zinc	8.96	3.0
Lactate	3.10	0.8	Choline	8.90	0.3
Citrate	3.13, 4.76, 6.40	3.0	Aluminium	5.00	0.7
Succinate	4.21, 5.64	0.4			
Acetate	4.76	1.3			
Others		31.4	Others		8.8

Table 8.5 Theoretical solubility and pH of salts of chlordiazepoxide

Salt	pK_a	Salt pH	Solubility (mg mL^{-1})
Base	4.80	8.30	2.0
Hydrochloride	−6.10	2.53	<165[a]
Maleate	1.92	3.36	57.1
Tartrate	3.00	3.90	17.9
Benzoate	4.20	4.50	6.0
Acetate [b]	4.76	4.78	4.1

[a] Maximum solubility of chlordiazepoxide hydrochloride, achieved at pH 2.89, is governed by crystal lattice energy and common ions.
[b] Chlordiazepoxide acetate may not form; pK_a of acetate too high and too close to that of drug ion.

ally be less hygroscopic and form less acidic or basic solutions (Table 8.5). Injections should ideally lie in the pH range 3–9 to prevent vessel or tissue damage and pain at the injection site. Oral syrups should not be too acidic, to enhance palatability. Packaging may also be susceptible: undue alkalinity will attack glass, and hydrochloride salts should not be used in aerosol cans as a propellant–acid reaction will corrode the canister.

From Table 8.5, not only does the intrinsic pH of the base solution fall significantly if salt forms are produced but, as a consequence, the solubility increases exponentially (Eqns 8.2 and 8.3). This has important implications in vivo. A weak base with an intrinsic solubility greater than 1 mg mL^{-1} will be freely soluble in the gastrointestinal tract, especially in the stomach. However, it is usually better to formulate with a salt, as it will control the pH of the diffusion layer (the saturated solution immediately adjacent to the dissolving surface, known as the pH microenviroment). For example, although chlordiazepoxide base (C_s = 2 mg mL^{-1} at pH_{sat} 8.3) meets the requirements for in vivo 'solubility' (Kaplan, 1972); commercial capsules contain chlordiazepoxide hydrochloride (C_s = 165 mg mL^{-1} at pH_{sat} 2.53).

A weak base will have a high dissolution rate in the stomach, but as it moves down the gastrointestinal tract the pH rises and dissolution rate falls. Conversely, a weak acid has minimal dissolution in the stomach but becomes more soluble and dissolution rate increases down the gut. Paradoxically, as dissolution rate increases so absorption falls because the drug is ionized.

The dissolution rate of a particular salt is usually much greater than that of the parent drug. Sodium and potassium salts of weak acids dissolve much more rapidly than do the parent acids, and some comparative data are shown in Table 8.6. On the basis of bulk pH these salts would be expected to have lower dissolution rates in the stomach. However, the pH of the diffusion layer (found by measuring the pH of a saturated bulk solution) is

higher than that of gastric fluid (which is approximately 1.5) because of its buffering action. The pH is the saturated unbuffered aqueous solution (calculated pH in Table 8.6) and the dissolution rate is governed by this pH and not the bulk medium pH.

In the intestine the salt does not depress the pH, unlike the acid which is neutralized, and the diffusion layer pH is again raised to promote dissolution. Providing that the acid forming the salt is strong, the pH of the solution adjacent to the dissolving surface will be that of the salt, whereas for the dissolving free base it will be the pH of the bulk dissolving medium. With weak bases, their salts dissolve rapidly in the stomach but there is no absorption, as the drug is ionized and absorption is delayed until the intestine. Any undissolved drug, as salt, rapidly dissolves, as the higher diffusion layer pH compensates for the higher bulk pH, which would be extremely unfavourable to the free base. Data for chlordiazepoxide are shown in Table 8.5. The maleate salt has a predicted solubility of 57 mg mL^{-1} but, more importantly, reduces the pH by 5 units. By controlling diffusion layer pH the dissolution rate can increase manyfold, independently of its position in the gastrointestinal tract. This is particularly important in the development of controlled-release products.

Different salts of a drug rarely change pharmacology, but only physical properties. This statement has been qualified to acknowledge that salts do affect the intensity of response. However, the salt form does change the physiochemical properties of the drug. Changes in dissolution rate and solubility affect the rate and extent of absorption (bioavailability), and changes on hygroscopicity and stability influence formulation.

Consequently each new drug candidate has to be examined to choose the most suitable salt, because each potential salt will behave differently and require separate preformulation screening. The regulatory authorities also treat each salt as a different chemical entity, particularly in the context of toxicity testing.

Solvents

It is generally necessary to formulate an injection even if there is no intention to market. The first-choice solvent is obviously water. However, although the drug may be freely soluble, it may be unstable in aqueous solution. Chlordiazepoxide HCl is such an example. Accordingly, water-miscible solvents are used:

1. in formulations to improve solubility or stability
2. in analysis to facilitate extraction and separation (e.g. chromatography).

Oils are used in emulsions, topicals (creams and ointments), intramuscular injections and liquid-fill oral preparations (soft and hard gelatin capsules) when aqueous pH and solvent solubility and stability are unattainable Table 8.7 shows a range of solvents to fulfil these needs.

Aqueous methanol is widely used in HPLC and is the standard solvent in sample extraction during analysis and stability testing. It is often made acidic or alkaline to increase solvent power and ensure consistent ionic conditions for UV analysis. Other pharmaceutical solvents are available but are generally only required in special cases. The most acceptable non-aqueous solvents pharmaceutically are glycerol, propylene glycol and ethanol. Generally for a lipophilic drug (i.e. a partition coefficient (log $P > 1$), solubility doubles through this series.

Where bulk is limited and the aqueous solubility is inadequate, it is better to measure the solubility in

Table 8.6 Dissolution rates of weak acids and their sodium salts

Drug	pK_a	pH (at C_s)	Dissolution rate (mg cm^{-2} min^{-1}) × 10^2	
			Dissolution media	
			0.1 M HCl (pH 1.5)	Phosphate (pH 6.8)
Salicylic acid	3.0	2.40	1.7	27
Sodium salicylate		8.78	1870	2500
Benzoic acid	4.2	2.88	2.1	14
Sodium benzoate		9.35	980	1770
Sulphathiazole	7.3	4.97	<0.1	0.5
Sodium sulphathiazole		10.75	550	810

Table 8.7 Recommended solvents for preformulation screening

Solvent	Dielectric constant (ϵ)	Solubility parameter (δ)	Application
Water	80	24.4	All
Methanol	32	14.7	Extraction, separation
0.1 M HCl (pH 1.1)			Dissolution (gastric), basic extraction
0.1 M NaOH (pH 13.1)			Acidic extraction
Buffer (pH 6–7)			Dissolution (intestinal)
Ethanol	24	12.7	Formulation
Propylene glycol	32	12.6	
Glycerol	43	16.5	
PEG 300 or 400	35		

aqueous solvent mixtures rather than in a pure organic solvent. Whereas solubilities at other levels and their mixtures can be predicted, the solubility in pure solvent is often inconsistent because of cosolvent effects. Furthermore, formulations rarely use pure non-aqueous solvent, particularly injections. For example, ethanol should only be used up to 10% in an injection to prevent haemolysis and pain at the injection site, and include isotonic salts.

Partition coefficient (K_w^o)

Partition coefficient (the solvent:water quotient of drug distribution) has a number of applications which are relevant to preformulation:

1. Solubility: both aqueous and in mixed solvents
2. Drug absorption in vivo: applied to a homologous series for structure activity relationships (SAR)
3. Partition chromatography: choice of column (HPLC) or plate (TLC) and choice of mobile phase (eluant).

Solvent solubility

The relative polarities of solvents can be scaled using dielectric constant (ε), solubility parameter (δ), interfacial (γ) and hydrophilic–lipophilic balance (HLB). The best solvent in any given application is one whose polarity matches that of the solute; an ideal, fully compatible solution exists when $\delta_{solvent} = \delta_{solute}$. This can be ascertained by determining solubility maxima, using a substituent contribution approach or the dielectric requirement of the system.

The most useful scale of polarity for a solute is K_w^o (oil:water partition coefficient), as the other approaches do not allow easy estimates for the behaviour of crystalline solids. For a wide range of drugs it is possible to relate solvent solubility and the partition coefficient ($\log K_w^o = \log P$). Yalkowsky and Roseman (1981) derived the following expression for 48 drugs in propylene glycol:

$$\log C_s = \log C_w + \int(0.89 \log P + 0.03) \quad (8.4)$$

Equation 8.4 can be applied more generally by introducing a factor ϕ to account for the relative solvent power of pharmaceutical solvents (see Table 8.8 for examples).

For a wide range of solvents Eqn 8.4 now becomes:

$$\log C_s = \log C_o + \int(\log \phi + 0.89 \log P + 0.03) \quad (8.5)$$

Methodology and structure activity prediction

Choice of non-aqueous solvent (oil) The oil:water partition (K_w^o) is a measure of the relative lipophilicity (oil-loving) nature of a compound, usually in the unionized state (HA or B), between an aqueous phase and an immiscible lipophilic solvent or oil.

Table 8.8 Solvent power (ϕ) of some pharmaceutical solvents

Solvent	Relative solvent power (ϕ)
Glycerol	0.5
Propylene glycol	1
PEG 300 or 400	1
Ethanol	2
DNA, DMF	4

Many partition solvents have been used. The largest database has been generated using *n*-octanol. The solubility parameter of octanol (δ = 10.24) lies midway in the range for drugs (8–12), although some non-polar ($\delta < 7$) and polar drugs ($\delta > 13$) are encountered. This allows measurable results between equal volumes of oil and aqueous phases.

In the ***shake flask method*** the drug, dissolved in one of the phases, is shaken with the other partitioning solvent for 30 minutes, allowed to stand for 5 minutes, and then the majority of the lower aqueous phase (density of octanol = 0.8258 g mL^{-1}) is run off and centrifuged for 60 minutes at 2000 rpm. The aqueous phase is assayed before (ΣC) and after partitioning (C_w) [the aqueous concentration] to give $K_w^o = (\Sigma C - C_w)/(C_w)$.

If the transfer of solute to the oil phase is small, ΔC_w is small, and any analytical error increases error in the estimate of K_w^o. Indeed, to encourage greater aqueous loss ($>\Delta C_w$) a considerably more polar solvent, *n*-butanol, has been used. Where the partition coefficient is high, it is usual to reduce the ratio of the oil phase from 1:1 to 1:4 or 1:9 in order to increase the aqueous concentration (C_w) to a measurable level.

For a 1:9 oil:water ratio $K_w^o = (10\ \Sigma C - C_w)/C_w)$.

The partition of a polar solute between an inert non-polar hydrocarbon e.g. hexane, and water is quite different from that of hydrogen bonding solvents such as octanol. The behaviour of the weak acid phenol (pK_a = 10) and weak base nicotine (pK_a = 3.1) is worthy of note. For phenol, $K_w^{octanol}$ = 29.5, whereas K_w^{hexane} = 0.11. The acidic solvent chloroform suppresses partition (K_w^o = 2.239), whereas ethyl acetate and diethyl ether are more polar. The basic behaviour of the solvents give higher K_w^o values. With solvents capable of both hydrogen donation and acceptance (octanol, nitrobenzene and oleyl alcohol), K_w^o is intermediate. For nicotine the behaviour is reversed, and the hydrogen donor (acidic) solvent chloroform partitions most strongly (K_w^o = 77.63), even though the neutral solvent nitrobenzene, which is marginally more lipophilic (log P = 1.87 against 1.96 for chloroform), gives similar values for both phenol and nicotine. Clearly both solute and solvent characteristics are important.

In general, polar solvents are advocated to correlate biological activity with physicochemical properties. Solvents less polar than octanol, measured by water solvency, have been termed hyperdiscriminating, whereas more polar solvents such as butanols and pentanols, are hypodiscriminating. This concept refers to the discriminating power of a partitioning solvent within a homologous series. With *n*-butanol the values of log P tend to be close, whereas with heptane and other inert hydrocarbons the differences in solute lipophilicities are exaggerated. *n*-Octanol generally gives a range consistent with other physicochemical properties when compared to drug absorption in the GI tract. Hyperdiscriminating solvents reflect more closely the transport across the blood–brain barrier, whereas hypodiscriminating solvents give values consistent with buccal absorption (Fig. 8.2). In rationalizing the effects of different partitioning solvents, a good correlation was found to exist between the solvent water content at saturation and solvent lipophilicity.

Certainly it is imperative to standardize on methodology, especially for the solvent. Where solubility constraints allow, this should be *n*-octanol, especially as the existing data bank is extensive.

Structure–activity relationships Since the pioneering work of Meyer and Overton numerous studies on correlating molecular structure and biological activity have been reported. These structure–activity relationships (SAR) can rationalize drug activity and, particularly in modern medicinal chemistry, facilitate a scientific approach to the design of more effective, elegant structural analogues.

The application of SAR depends on a sound knowledge of the physicochemical properties of each new drug candidate in a therapeutic class, and preformulation is an essential information source.

It is assumed in SAR that:

$$\log K_w^o = a \times \log k_w^{octanol} + b \qquad (8.6)$$

This relationship holds for all polar and semipolar solvents, but with non-polar solvents (hexane to isooctane) correlations are poor, and this seems to be related to water content. Given the importance of water, it is imperative that the octanol is saturated with the aqueous phase and the aqueous phase with octanol prior to any determination, otherwise the partitioning behaviour of the drug will be confounded by the mutual partitioning of the two solvents.

Although the aqueous phase is often water, it is better to measure log P under controlled pH. All drugs capable of ionization and with a measurable pK_a will have intrinsic buffer capacity that affects the aqueous pH. Depending on the degree of dissociation, this will lead to an apparent K_w^o rather than the true (absolute) value, when the drug is unionized.

Because the ionized species will have greater aqueous solubility and lower lipophilicity to HA or BOH, the measured K_w^o (apparent) will be inevitably lower. Accordingly, K_w^o (true) should be measured at >2 pH units away from the pK_a ($pK_a - 2$ (acid); $pK_a + 2$ (base)) and the aqueous phase should contain a

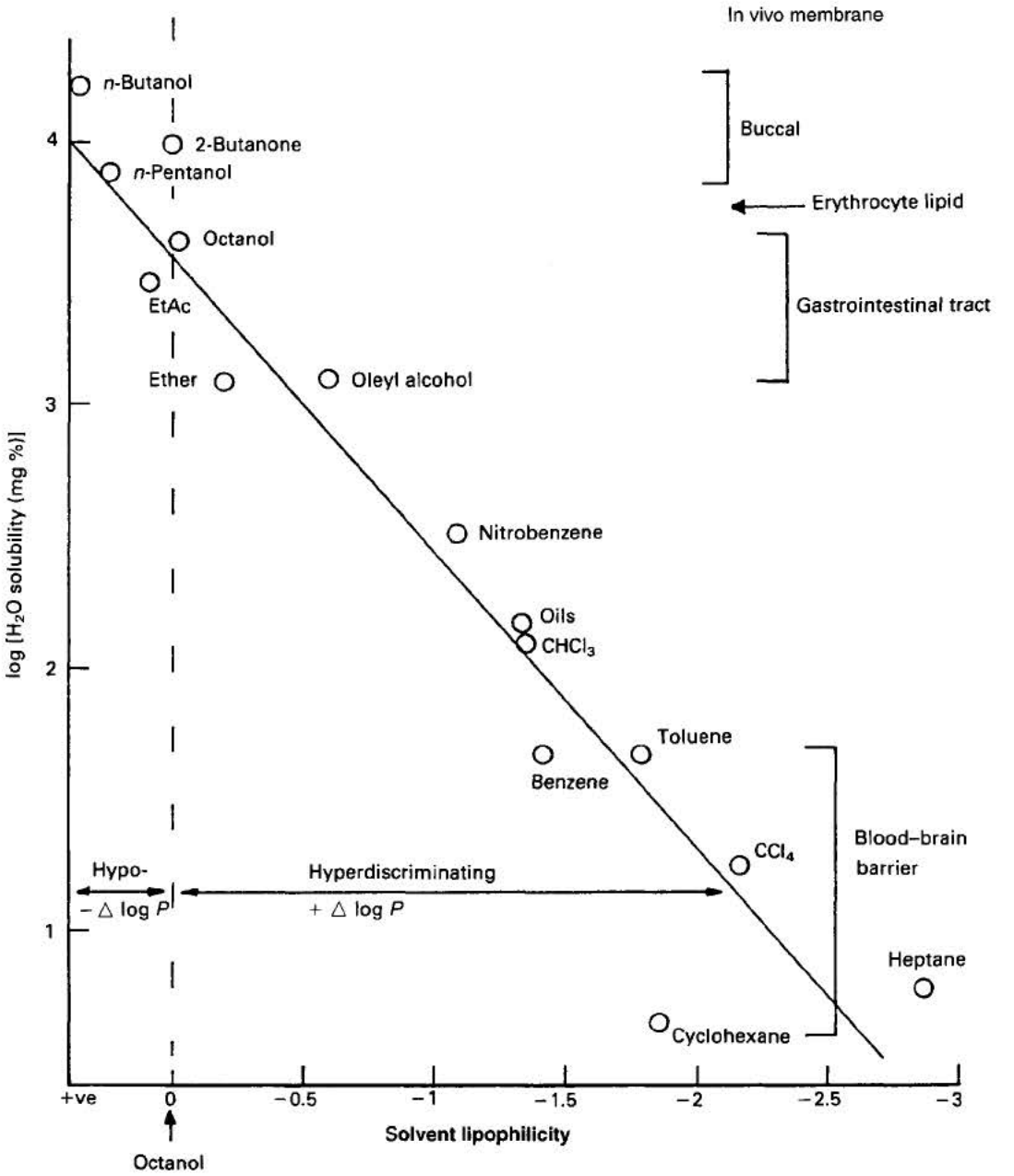

Fig. 8.2 Discriminating power of partitioning solvents as a function of their water capacity

suitable buffer. Given the importance of log P (log K_w^o) in SAR, comparative data generated in a therapeutic class (R_nX, where X is the therapeutic nucleus and n is a number of substituents R) should also be determined at physiological pH 7.4.

Quantitative SAR (QSAR) is based on the premise that drug absorption is a multipartitioning process (repeated adsorption and desorption) across cellular membranes and dependent on the lipophilicity of the drug, and the rate of penetration is proportional to the drug partition coefficient in vitro. Clearly the ionic condition in vivo will affect any correlation and, accordingly, for dissociating drugs the in vitro conditions should be similar. The widespread use of octanol in these studies and the existence of many excellent correlations in vivo is probably not

fortuitous. Octanol exhibits hydrogen bonding acceptor and donor properties typical of many biological macromolecules. The partial polarity of octanol allows the inclusion of water, which is also a feature of biological lipid membranes and leads to a more complex partitioning behaviour than a less polar, essentially anhydrous solvent.

The effect of salt formation on the measured log P is shown in Table 8.9. Generally the log P differs between 3 and 4 (K_w^o from 1000 to 10 000). The lipophilicity falls by three to four orders of magnitude, which accounts for the significant increase in solubility of the salt. The physicochemical model for biological activity assumes that activity of a compound is related to the following factors associated with molecular structure:

- Electronic (charge)
- Steric (spatial size)
- Hydrophobic effects (partitioning).

Account must also be taken of structural and theoretical aspects, so that:

$$\text{Biological activity} = f\,[(\text{electronic}) + (\text{steric}) + (\text{hydrophobic}) + \text{structural/theoretical}) \quad (8.7)$$

The electronic parameter in Eqn. 8.7 is quantified by the sigma (σ) substituent constant of Hammett and reflects chemical reactivity in a homologous series. The substituent constant (σ) is positive for electron withdrawal (acids), whereas electron donor groups (bases) give a negative value. It can be used to predict pK_a (Perrin et al 1981).

Steric effects occur when there is a direct interaction between the substituent and the parent nucleus, and is related to substituent bulk. High positive values of the steric effect parameter, E_s, indicate significant steric effects, with intra- and intermolecular hindrance to a reaction or finding at the active site.

Table 8.9 The effect of salt formation on the log *P* of some weakly basic drugs

Free base and hydrochloride salt	log *P*	Δlog *P*
Chlorpromazine	5.35	3.84
Chlorpromazine HCl	1.51	
Promazine	4.49	3.58
Promazine HCl	0.91	
Trifluopromazine	5.19	4.28
Trifluopromazine HCl	1.78	
Trifluoperazine	5.01	3.34
Trifluoperazine HCl	1.69	
Diphenylhydramine	3.30	3.42
Diphenylhydramine HCl	−0.12	

The hydrophobic component is measured by the distribution between an aqueous phase and an immiscible lipid phase and parallels drug adsorption and distribution in vivo. A relationship between partition coefficients has been demonstrated within a series by quantifying differences using an additive substituent constant (π). The constant is related almost completely to the effect of a particular substituent and much less to the parent compound, and this allows the prediction of the partition coefficient, log P, of a new derivative with reasonable accuracy. In addition, π can be related to biological effect as it is an additive component of the partition coefficient, and this has led to the wider application of SAR, with other modifications, notably taking into account of aromatic electron density and steric effects, to give ***quantitative*** SAR (i.e. QSAR).

Although all these substituents are useful, log P remains the most useful physical parameter and undoubtedly the most reliable data and correlations still come from experimentally derived partition values for the analogues in a series.

Dissolution

The dissolution rate of a drug is only important where it is the rate-limiting step in the absorption process. Kaplan (1972) suggested that provided the solubility of a drug exceeded 10 mg mL^{-1} at pH <7, no bioavailability- or dissolution-related problems were to be expected. Below 1 mg mL^{-1} such problems were quite possible, and salt formation could improve absorption and solubility by controlling the pH of the microenvironment independently of the drug and dosage forms' position within the GI tract.

Intrinsic dissolution rate

When dissolution is controlled solely by diffusion (transport control), the rate of diffusion is directly proportional to the saturated concentration of the drug in solution (i.e. solubility). Under these conditions the rate constant K_1 is defined by:

$$K_1 = 0.62\, D^{2/3}\, \nu^{1/6}\, \omega^{1/2} \quad (8.8)$$

where ν is the kinematic viscosity and ω is the angular velocity of a rotating disc of drug. By maintaining the dissolution fluid viscosity and rotational speed of the sample constant, the dissolution rate

(dc/dt) from a constant surface area (A) will be constant and related solely to solubility. Under sink conditions ($C_s >>> C$) gives:

$$dc / dt = \frac{A}{V} K_1 C_s \quad (8.9)$$

Intrinsic dissolution rate (IDR) is given by:

$$\text{IDR} = K_1 C_s \text{ (mg cm}^2 \text{ min}^{-1}\text{)} \quad (8.10)$$

This constant rate differs from the dissolution from conventional dosage forms, which is known as total dissolution (mg mL^{-1}), where the exposed surface area (A) is uncontrolled as disintegration, deaggregation and dissolution proceed. Accordingly, the IDR is independent of formulation effects and measures the intrinsic properties of the drug and salts as a function of dissolution media, e.g. pH, ionic strength and counter-ions.

Measurement of intrinsic dissolution rate A compressed disc of material can be made by slow compression of 500 mg of drug in a 13 mm IR disc punch and die set to a high compaction pressure greater than 500 MPa (to ensure zero porosity) and a long dwell time (to improve compaction). The metal surfaces in contact should be prelubricated with, for example, stearic acid (5% w/v in chloroform). The compressed disc is fixed to the holder of the rotating basket apparatus using a low-melting paraffin wax and successively dipped so that the top and sides of the disc are coated. The lower circular face should be cleared of residual wax using a scalpel and carefully scraped to remove any stearic acid transferred from the punch face.

The coated disc is rotated at 100 rpm, 20 mm from the bottom of a 1 L flat-bottomed dissolution flask containing 1 L of fluid at 37°C. The amount of drug release is then monitored, usually by UV spectrometry, with time. The slope of the line divided by the exposed surface area gives the IDR (mg cm^2 min^{-1}).

Each candidate should be measured in 0.05 M HCl (gastric) and phosphate buffer pH 7 (intestinal), and distilled water especially if sink conditions are not possible for a weak base at pH 7 or a weak acid in 0.05 M HCl. Sink conditions maintain the bulk concentration (C) at a low level otherwise the rate of dissolution is progressively reduced and the plot of concentration against time becomes nonlinear. It is recommended that C should not exceed $0.1C_S$.

By comparing the IDR of a salt in water with that obtained in acid and alkali, or the free base with its salts in the same medium, a measure of the salt's ability to control its immediate microenvironment will emerge.

The equation derived from the Henderson–Hasselbalch equation:

$$\text{IDR} = k' [C_o(1 + \text{antilog } [pK_a - \text{pH}])] \quad (8.11)$$

shows that the rate of dissolution of a drug candidate is clearly a function of its intrinsic solution (C_o), its dissociation constant (pK_a), and the pH of either the bulk dissolution medium or the microenvironment created by the dissolving salt. Using the measured rate of the free base at known bulk pH, expected rates in other media, using the experimental salts, can be calculated and compared with experimental values.

The importance of improvements in the IDR due to microenvironmental pH control lies in the improvement in vivo of a salt over the parent drug. Where no increase is found there is likely to be no advantage in using that particular salt. Improvements are obviously more likely if the salt former is strong. For a weak base, the hydrochloride ($pK_a = -6.10$) offers the best advantage, but in some instances this may prove disappointing because of Cl^- ions. Thus the measurement of IDR can be useful diagnostically.

Common ion effect

An often overlooked interaction is the common ion effect. A common ion often significantly reduces the solubility of a slightly soluble electrolyte. The 'salting out' results from the removal of water molecules as solvent owing to the competing hydration of other ions. The reverse process, 'salting in', arises with larger anions, e.g. benzoate, salicylate, which open the water structure. These hydrotropes increase the solubility of poorly water-soluble compounds such as diazepam.

Hydrochloride salts often exhibit suboptimal solubility in gastric juice owing to the abundance of Cl^- ions (Table 8.10). Counter-ions other than Cl^-, such

Table 8.10 Examples of weakly basic drugs which have decreased solubility in acidic and Cl^- solutions

Chlortetracycline
Demethylchlortetracycline
Methacycline
Demeclocyline
Phenazopyridine
Cyproheptadine
Bromhexine
Triamterine

as nitrate, sulphate and phosphate, have also been implicated. They are usually inorganic, because of their small size.

To identify a common ion interaction the IDR of the hydrochloride (or inorganic) salt should be compared between:

- water and water containing 1.2% w/v NaCl, and
- 0.05 M HCl and 0.9% w/v NaCl in 0.05 M HCl.

Both saline media contain 0.2M Cl^-, which is typically encountered in fluids in vivo.

A common ion effect with Cl^- will result in a significantly reduced IDR in the presence of sodium chloride. Other salt forms are then indicated, e.g. tosylate, mesylate etc., but the parent molecule will still remain sensitive to Cl^- and solubilities will be suppressed in the presence of saline, although not to the same extent as Cl^- is not involved in the dissolving microenvironment. Any improvement with the new salt can be assessed by again measuring the IDR with and without saline. As some compounds are sensitive to other counter-ions, e.g. nitrate, sulphate and phosphate can be demonstrated by including the appropriate sodium salt in the dissolution medium. Phase solubility studies have indicated that basic amine drugs are more soluble in organic acids than inorganic. Where a hydrochloride salt exhibits suboptimal solubility then the next logical choice is probably a salt of toluene sulphonic acid (tosylate: $pK_a = -1.34$). Mesylate, napsylate, besylate and maleate salts offer progressively more weaker acidic alternatives.

With low-solubility amine drugs, the salts of polyhydroxy acids, e.g. lactate, often give the greatest aqueous solubility because of their accessible hydroxy groupings.

MELTING POINT

Techniques

The melting point of a drug can be measured using three techniques:

1. Capillary melting
2. Hot stage microscopy
3. Differential scanning calorimetry or thermal analysis.

Capillary melting

Capillary melting (the observation of melting in a capillary tube in a heated metal block) gives information about the melting range but it is difficult to assign an accurate melting point.

Hot stage microscopy

This is the visual observation of melting under a microscope equipped with a heated and lagged sample stage. The heating rate is controllable and up to three transitions can be registered. It is more precise as the phase transitions (first melt, 50% melt and completion) can be registered on a recorder as the melting proceeds, and because of the high magnification the values are more accurate.

Differential scanning calorimetry and thermal analysis

Neither of the previous methods is as versatile as either differential thermal analysis (DTA) or differential scanning calorimetry (DSC). An additional advantage is that the sample size required is only 2–5 mg.

DTA measures the temperature difference between the sample and a reference as a function of temperature or time when heating at a constant rate. DSC is similar to DTA, except that the instrument measures the amount of energy required to keep the sample at the same temperature as the reference, i.e. it measures the enthalpy of transition.

When no physical or chemical change occurs within the sample then there is neither a temperature change nor input energy to maintain an isotherm. However, when phase changes occur then latent heat suppresses a temperature change and the isothermal energy required registers as an electrical signal generated by thermocouples. Crystalline transitions, fusion, evaporation and sublimation are obvious changes in state which can be quantified (Fig. 8.3).

The major concern in preformulation is polymorphism, and the measurement of melting point and other phase changes is the primary diagnostic tool. Confirmation by IR spectroscopy and X-ray diffraction is usually required.

Polymorphism

A polymorph is a solid material with at least two different molecular arrangements that give distinct crystal species. These differences disappear in the liquid or the vapour state. Of concern are their relative stabilities and solubility. The highest-melting species is generally stable; other polymorphs are metastable and convert to the stable form. There are also potentially large differences in their physical

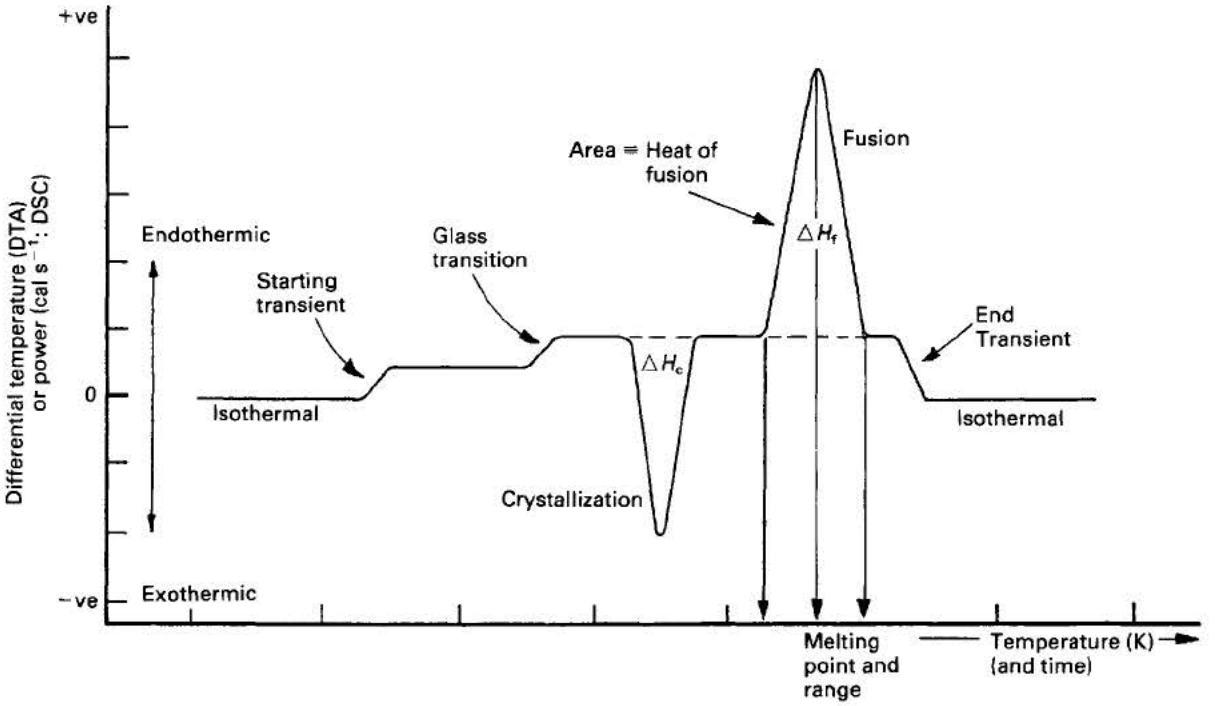

Fig. 8.3 Schematic differential scanning calorimeter thermogram.

properties so that they behave as distinct chemical entities. Solubility (particularly important in suspensions and biopharmaceutically), melting point, density, crystal shape, optical and electrical properties and vapour pressure are often very different for each polymorph.

Polymorphism is remarkably common, particularly within certain structural groups: 63% of barbiturates, 67% of steroids and 40% of sulphonamides exhibit polymorphism.

The steroid progesterone has five polymorphs, whereas the sulphonamide sulphabenzamide has four polymorphs and three solvates. The importance of polymorphism is illustrated by the biopharmaceutical data for fluprednisolone (Fig. 8.4).

It is convention to number the polymorphs in order of stability at room temperature, starting with form I using Roman numerals. Form I usually has the highest melting point and the lowest solubility; in suspension formulation it is essential to use the least soluble polymorph because of Ostwald ripening.

Accordingly, in preformulation the following should be considered.

- How many polymorphs exist?
- How stable are the metastable forms?
- Is there an amorphous glass?
- Can the metastable forms be stabilized?
- What is the solubility of each form?
- Will a more soluble form survive processing and storage?

Pseudopolymorphism (solvates)

Prior to this, the presence of solvates or false polymorphs, sometimes (incorrectly and confusingly) called pseudopolymorphs, should be identified, as most polymorphs can be obtained by changing the recrystallizing solvent. Typical solvents inducing polymorphic change are water, methanol, ethanol, acetone, chloroform, *n*-propanol, isopropanol alcohol, *n*-butanol, *n*-pentanol, toluene and benzene. Trace levels of solvent are usual in early batches of new drug candidates (residues from the final crystallization). These can become molecular additions to the crystal and change habit. These hydrates (water) and solvates (e.g. methanolate, ethanolate) have been confused with true polymorphism and have led to the term pseudopolymorphism.

The distinction between these false forms and true polymorphs can be ascertained by observing the melting behaviour of the compound dispersed in silicone oil using hot-stage microscopy.

Pseudopolymorphs will evolve a gas (steam or solvent vapour), causing the oil to bubble. True

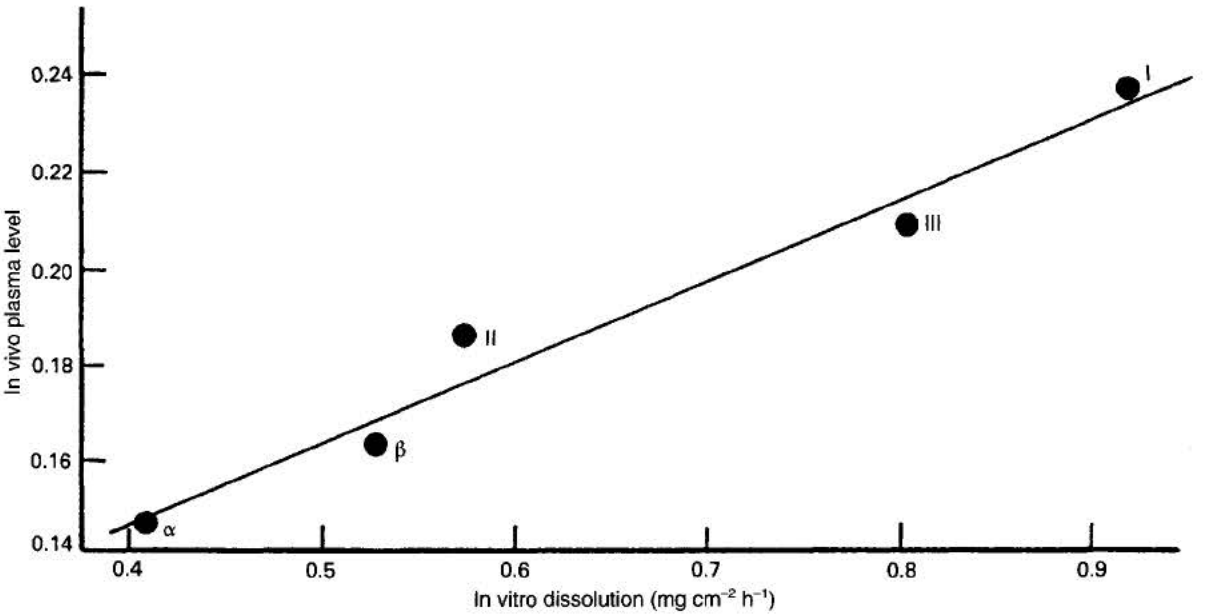

Fig. 8.4 The relationship between in vitro and in vivo release from fluprednisolone implants.

polymorphs merely melt, forming a second globular phase. The temperature at which the solvent volatilizes will be close to the boiling point of the solvent.

True polymorphism

After the study of pseudopolymorphism (see also Chapter 9 for further discussion on polymorphism), the evaluation of true polymorphism can proceed unconfounded. Most polymorphs are obtained by solvent manipulation. Others can be produced without the presence of solvent by thermal techniques, notably sublimation and recrystallization from the melt. Supercooling of the melt is particularly useful in discovering unstable modifications.

The initial difficulty is to measure the melting point of the metastable form, and here heating rate is critical. Too-rapid heating will obscure the endotherm, whereas too slow a heating rate may allow transition or encourage decomposition. Often, therefore, comparison at two rates, e.g. 2 and 20°C min^{-1}, is necessary.

The difference in melting point (ΔT_m) between polymorphs is a measure of the metastable polymorph stability. Where $\Delta T_m < 1$°C then neither is significantly more stable and either may be obtained upon conventional crystallization. If ΔT_m is 25–50°C then the lower melting species will be difficult to crystallize and will rapidly revert. The closer the two melting points ($\Delta T_m \rightarrow$ 1–25°C) then the unstable form(s) can be obtained easily before a solid–solid transformation occurs. This can be suppressed by using small samples, as individual crystals of even highly unstable forms can be melted.

If it appears that polymorphism is occurring or likely, then a cooperative study with the bulk chemists should determine the most stable form (chemical and physical). Differences in solubility and melting point must also be assessed and then a decision made to determine which form to progress through to the next stage of formulation. Small differences in stability but higher solubility of a relatively metastable form may lead to a preferential choice of a polymorph other than form I, but this is unlikely and is not encouraged by regulatory authorities.

Crystal purity

Thermal analysis has been widely used as a method of purity determination and the USP includes an appendix describing the methods. This is particularly pertinent at the preformulation stage, because early samples of a new drug are inevitably 'dirty' while improvements in synthetic route are made. Thermal analysis is rapid and will discriminate 0.002 mole% of impurity.

Solubility

The most important reason to determine melting point during preformulation is crystalline solubility.

Such studies are particularly important because the scarcity of available drug powder often precludes accurate solubility determinations.

Melting point and solubility are related via the latent heat of fusion, which is the amount of heat generated during melting or fusion. A crystal with weak bonds has a low melting point and low heat of fusion. Conversely, a strong crystal lattice leads to a high melting point and a high heat of fusion. Because solubility also requires the disruption of crystal structure to allow molecular dispersion in the solvent, it is also influenced by intermolecular forces.

Polymorphs differ in melting point and solubility. The existence of different crystal arrangements for the same compound inevitably leads to differences in crystal lattice energy, as intermolecular distances will be different in the alternative forms. This effect is shown in Figure 8.5 for riboflavine.

ASSAY DEVELOPMENT

The assumption that the drug is stable may not be valid as drugs are notoriously unstable, particularly as hydrolysis is often the predominant cause. In order to follow drug stability, in both solution and solid phase, it is mandatory to have suitable stability-indicating assays. In some cases UV spectroscopy can be used, but in general chromatography is required to separate the drug from its degradation products and any excipients. Thin-layer

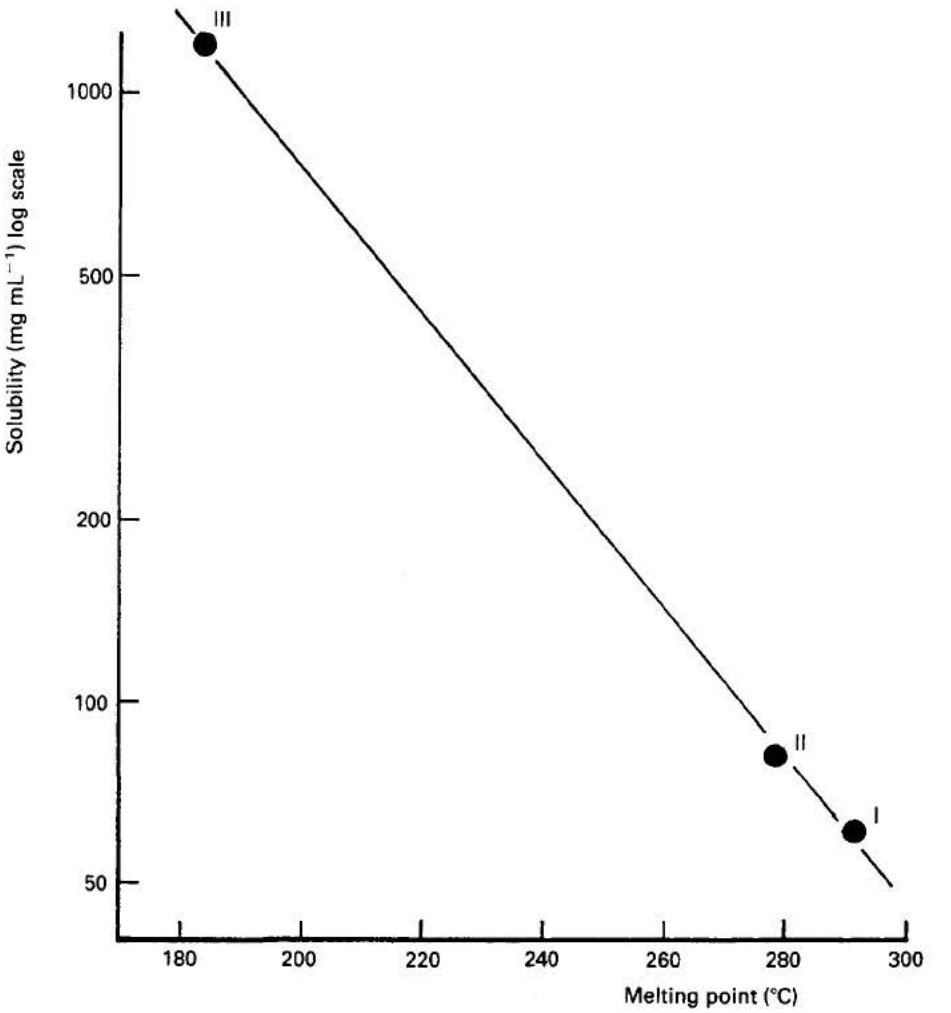

Fig. 8.5 The relationship between melting point and solubility for three polymorphs of riboflavine.

chromatography (TLC) is widely used in a semi-quantitative mode to estimate impurity levels, to establish the number of impurities, and to collect samples from the plate for subsequent HPLC (high-performance (pressure) liquid chromatography. HPLC is now acknowledged as the most versatile and powerful technique in pharmaceutical analysis and is the method of choice in preformulation stability assessment.

UV spectroscopy

The principles of UV spectroscopy were enumerated earlier and will serve to quantify many of the subsequent physical constants and solubilities above. TLC, and particularly HPLC, has largely superseded UV spectroscopy as a basic analytical tool in stability assessment. However, certain UV techniques are worthy of discussion:

- Solubility
- Molecular weight
- pK_a
- Assay (potency)
- Mixtures:
  - resolving compound products
  - stability: hydrolysis, oxidation (coloured products).

Molecular weight

To a first approximation the molar extinction coefficient (e) of a chromophore (absorbing molecular group) is unaffected by distant substituent groups in a molecule. Therefore, if e of the chromophore is known from another related compound in the series, the molecular weight of the new derivative can be calculated from the absorbance of a solution of known concentration, as mol. wt. = $10\ e/E$.

pK_a

pK_a measurement by spectroscopy is indicated when solubility is too low, or when the pK_a is particularly low (<2) or high (>11) for determination by potentiometry. The method depends on determining the ratio of molecular species (neutral molecular at pH < $pK_a - 2$ (acid), pH > $pK_a + 2$ (base)) to the ionized species in a series of seven buffers (pH = ±1 of pK_a). An analytical wavelength (λ) is chosen where the greatest difference in absorbance exists between the molecular species and the pure ionized moiety at two pH units from the pK_a.

Thin-layer chromatography

All chromatographic procedures emanate from the Russian botanist Tswett (1906), who separated plant pigments by pipetting solutions on to the top of packed glass tubes. Tswett's work led to the development of column chromatography and, under pressure, to HPLC. Other workers used paper as a support, and before the advent of HPLC paper chromatography was used for simplicity and speed. TLC arose from a need to satisfactorily separate lipids, which paper techniques could not achieve, but it was soon realized that the technique was also considerably more flexible. Paper chromatography is limited by the cellulose support, whereas the thin layer of material on a glass plate can be prepared from a slurry of a wide variety of different chemical types, e.g. silica gel, celite, alumina, cellulose (analogous to paper chromatography) and chemically modified celluloses; and more recently with the advent of reversed-phase chromatography, C_2, C_8 and C_{18} silanized and diphenyl silica.

Modern TLC was established by Stahl (1956) and separations were found to be considerably shorter than on paper, spots were more compact, resolution better, and submicrogram samples could be separated and recovered if necessary (by scraping away the spot using a fine spatula), re-extracted and injected on to HPLC.

TLC is now generally regarded as a reliable and sensitive qualitative technique for the separation of complex mixtures in stability samples. In a typical analysis, extracted samples are spotted 20 mm from the bottom of a square glass plate, 200 × 200 mm, coated with a dry slurry of silica (250 μm thick) and placed in a closed tank containing a 10 mm layer of eluting solvent, which has produced a saturated vapour phase. The sample is developed (separated) by the capillary movement of the solvent up the plate and is therefore similar to HPLC. It is a thin flat column (stationary phase) with solvent (mobile phase) pumped by capillary flux, and much of the theory (Snyder 1968) is the same. Consequently, TLC and HPLC are complementary. TLC will quantify the number of components (as they can be seen) and estimate their concentration by reference to standards run concurrently, whereas HPLC can quantify their level, confident that all have been separated. The developing solvent for TLC (particularly HPTLC (high-performance TLC) and reverse phase) is also a useful guide to identify the mobile phase for HPLC.

High-performance liquid chromatography

High-performance (pressure) liquid chromatography (HPLC) is essentially column chromatography

performed by eluting under pressure. By pumping the eluting solvent (mobile phase) under a pressure of up to 40 MPa (6000 psi) and a flow rate of up to 3 mL min^{-1}, the column can be much smaller and use much smaller particle size packing material (stationary phase). This results in shorter retention times (solute time on column), high sensitivity (typically 1 ng), the need for only a small sample volume (0–50 μL) and yet high selectivity (separation power) for the resolution of complex mixtures.

HPLC methods can be divided into two distinct modes.

Normal-phase HPLC

Normal phase HPLC is performed by eluting a silica-packed column, which is hydrophilic, with a non-polar mobile phase. The mobile phase is usually hexane, to which is added one or more of the following, in increasing order of polarity: chloroform ($CHCl_3$), tetrahydrofuran (THF), acetonitrile (ACN), isopropyl alcohol (IPA) or methanol (MeOH). Separation is achieved by partition with differential adsorption and desorption of both the solute and solvents during passage down the column. Polar solutes are retained, but more lipophilic molecules are not. By increasing the polarity of the mobile phase (e.g. by adding MeOH or IPA), polar solutes are eluted more quickly, whereas non-polar solutes are better retained and their order of retention is changed. Decreasing solvent polarity increases polar solute retention and facilitates the elution of lipophilic molecules.

In general, normal-phase HPLC is used for moderately polar solutes (freely soluble in methanol). Non-polar hydrocarbon-soluble solutes are difficult to retain and very polar and water-soluble solutes are difficult to elute sufficiently.

Reverse-phase HPLC

When the solute is eluted by a polar (largely aqueous) mobile phase over a hydrophobic stationary phase, the chromatography is known as reverse phase. Solute behaviour is the reverse of that described for normal phase HPLC, which uses a hydrophilic silica stationary phase. Separation between the stationary phase and the mobile phase is solvophobic, analogous to partitioning.

Hydrophobicity of the stationary phase is achieved by bonding a coating on to the silica support. The most common bonded phases are alkyl silanes of C_{18} (octodecysiolane, ODS), C_8 (octysilane, OS), and C_1 (trimethysilane). The predominantly aqueous mobile phase usually contains methanol, ACN and/or THF to modify solvent polarity by matching the lipophilicity of the solutes in order to facilitate good chromatography. Ionization control can be achieved in the range pH 2–8. The inclusion of 1–2% acetic acid or diethylamine is used to suppress the ionization of weak acids and bases respectively (***ion-suppression chromatography*** is used to increase lipophilicity and improve the retention of polar solutes).

In general polar solutes have short retention times on reverse phase, whereas non-polar compounds are retained. Increasing the mobile phase polarity (by increasing the water concentration) shortens retention for polar solutes while retaining fewer polar compounds. Decreasing solvent polarity (by decreasing water concentration) helps retain polar compounds, but more lipophilic solutes are eluted more rapidly. Non-aqueous reverse phase (NARP HPLC, where THF or methylene chloride replaces water in the mobile phase) is used to separate lipophilic solutes.

The great flexibility of choice in mobile phase (by using solvents ranging from water to hexane), the increasing number of available stationary phases (particularly bonded phases) and the inherent sensitivity of HPLC produces a powerful analytical technique. It is the method of choice in preformulation stability studies.

DRUG AND PRODUCT STABILITY

Wherever possible, commercial pharmaceutical products should have a shelf-life of 3 years. The potency should not fall below 95% under the recommended storage conditions and the product should still look and perform as it did when first manufactured.

By investigating the intrinsic stability of the drug it is possible to advise on formulation approaches and indicate types of excipient, specific protective additives and packaging which are likely to improve the integrity of the drug and product. Typical stress conditions are shown in Table 8.11.

Drug degradation occurs by four main processes:

- Hydrolysis
- Oxidation
- Photolysis
- Trace metal catalysis.

Hydrolysis and oxidation are the most common pathways, and in general light (c) and metal ions catalyse a subsequent oxidative process.

Table 8.11 Stress conditions used in preformulation stability assessment

Test	Conditions
Solid	
Heat (°C)	4, 20, 30, 40, 40/75% RH, 50 and 75
Moisture uptake	30, 45, 60, 75 and 90% RH at RT[a,b]
Physical stress	Ball milling
Aqueous solution	
pH	1, 3, 5, 7, 9 and 11 at RT and 37°C. Reflux in 1 M HCl and 1 M NaOH
Light[c]	UV (254 and 366 nm) and visible (south-facing window) at RT
Oxidation[c]	Sparging with oxygen at RT; UV may accelerate breakdown

[a] RT is ambient room temperature. Can vary between 15 and 25°C.
[b] Saturated solutions of $MgBr_2$, KNO_2, NaBr, NaCl and KNO_3 respectively.
[c] At pH of maximum stability in simple aqueous solution.

Temperature

Thermal effects are superimposed on all four chemical processes. Typically a 10°C increase in temperature can produce a 2–5-fold increase in decay. Often the increase in reaction rate with temperature follows an Arrhenius-type relationship: a plot of the log of the rate of reaction against the reciprocal of absolute temperature yields a straight line. The reaction rate can then be calculated at any temperature and allows a prediction of shelf-life at room temperature by extrapolation. This assumption forms the basis of accelerated stability tests. However, the mechanism or pathway of the chemical breakdown often changes with temperature. This will be indicated by a discontinuity or 'knee joint' in the Arrhenius plot. This is not easily detected and would inevitably lead to erroneous conclusions, based on elevated temperature data, to predict shelf-lives at room temperature or under refrigeration. Reactions often change at about 50°C, and this is a sensible ceiling.

Order of reaction

The time course of degradation depends on the number of reactants, whose concentration influences the rate. It is often more convenient to express reaction rates in terms of time. The most common is the ***half-life***, the time at which the concentration has halved ($t_{1/2}$ or t_{50}). The shelf-life of a product can be likewise expressed as t_{95} (i.e. the time for 5% loss) etc.

In the absence of a definitive value for the activation energy (E_a), which can be obtained from the slope of the Arrhenius plot, it is prudent to assume a low value (e.g. 10 kcal mol^{-1}), as this will lead to higher reaction rates and any prediction of shelf-life will be conservative. Values for a wide range of drug degradation reactions are 10–100 kcal mol^{-1}, but are usually in the range 15–60 kcal mol^{-1} with a mean of 19.8. Most occur by first-order kinetics (logarithmic) but some are zero order, e.g. aspirin in aqueous suspension, and a few are second order, e.g. chlorbutol hydrolysis.

Hydrolysis

The most likely cause of drug instability is hydrolysis. Water plays a dominant role and in many cases it is implicated passively as a solvent vector between two reacting species in solution. The solution is often saturated, so that studies in dilute solution can be completely misleading (see Solid-state stability, later in this chapter).

Hydrolytic reactions involve nucleophilic attack of labile bonds, e.g. lactam > ester > amide > imide, by water on the drug in solution, and are first order. When this attack is by a solvent other than water it is known as ***solvolysis***.

A number of conditions catalyse the breakdown:

- The presence of OH^-
- The presence of H_3O^+
- The presence of divalent metal ions
- Ionic hydrolysis (protolysis) is quicker than molecular
- Heat
- Light
- Solution polarity and ionic strength
- High drug concentrations.

The influence of pH

The degradation of most drugs is catalysed by extremes of pH, i.e. high $[H_3O^+]$ and $[OH^-]$, and many drugs are most stable between pH 4 and 8.

Where maximum stability dictates wider values, it is important for injections that there is low buffer capacity to prevent unnecessary challenge to the homeostatic pH (7.4) of blood.

Weakly acidic and basic drugs are most soluble when ionized, and it is then that instability is most likely as they are charged. This leads to a problem, as many potent drugs are extremely poorly soluble and pH ionization is the most obvious method to obtain a solution. In some cases, therefore, the inclusion of a water-miscible solvent in the formulation will increase stability by:

1. Suppressing ionization
2. Reducing the extreme of pH required to achieve solubility
3. Reducing water activity by reducing the polarity of the solvent, e.g. 20% propylene glycol in chlordiazepoxide HCl injection.

Reactions in aqueous solution are usually catalysed by pH, and this is monitored by measuring degradation rates (usually pseudo first order) against pH, keeping temperature, ionic strength and solvent concentration constant. Suitable buffers include acetate, citrate, lactate, phosphate and ascorbate (an intrinsic antioxidant).

Solvolysis

Where the reacting solvent is not water, then breakdown is termed solvolysis. Furthermore, the definition can be extended to include any change in solvent polarity (usually measured as dielectric constant) as a result of increased ionic strength. Phenobarbitone is considerably more stable in preparations containing water-miscible solvents, whereas aspirin, which undergoes extensive hydrolysis, is degraded further by aqueous solvents. Both effects are directly related to the dielectric constant (polarity) of the solvent. In general, if a compound produces degradation products which are more polar then the addition of a less polar solvent will stabilize the formulation. If the degradation products are less polar, then the vehicle should be more polar to improve stability. With the hydrolysis of neutral non-polar drugs, e.g. steroids, the transition state will be non-polar with no net charge. In this case solvents will not affect the rate of decomposition and can be used with impunity to increase solubility.

Oxidation

Oxidation is controlled by the environment, i.e. light, trace metals, oxygen and oxidizing agents. Reduction is a complimentary reaction (redox) and there is a mutual exchange of electrons. Oxidation is a loss of electrons and an oxidizing agent must be able to take electrons. In organic chemistry, oxidation is synonymous with dehydrogenation (the loss of hydrogen) and this is the mode of action of polyhydroxphenol antioxidants, e.g. hydroquinone. However, most antioxidants function by providing electrons or labile H^+, which will be accepted by any free radical to terminate the chain reaction. A prerequisite for effective antioxidant activity in any particular preparation is that the antioxidant is more readily oxidized than the drug.

Chelating agents

Chelating agents are complexes, unlike simple ligands, e.g. ferrocyanide ($Fe(CN_6^{4-})$, which form complex salts by a single bond provided by a lone electron pair. Chelating agents are capable of forming more than one bond. For example, ethylene diamine is bidentate (two links), tripyridyl is tridentate (three) and ethylene diamine tetra-acetic acid (EDTA) is hexadentate (six), which makes it particularly effective as a pharmaceutical chelating agent.

Photolysis

Oxidation, and to some extent hydrolysis, is often catalysed by light. The energy associated with this radiation increases as wavelength decreases, so that the energy of UV visible is greater than that of IR and is independent of temperature (Table 8.12).

When molecules are exposed to electromagnetic radiation they absorb light (photons) at characteristic wavelengths which causes an increase in energy, which can:

- cause decomposition
- be retained or transferred
- be converted to heat
- result in light emission at a new wavelength (fluorescence, phosphorescence).

Table 8.12 Relationship between wavelength and associated energy of various forms of light

Type of radiation	Wavelength (nm)	Energy (kcal mol^{-1})
UV	50–400	287–72
Visible	400–750	72–36
IR	750–10 000	36–1

Natural sunlight lies in the wavelength range 290–780 nm, of which only the higher energy (UV) range (290–320 nm) causes photodegradation of drugs, and sunburn. Fluorescent lighting tubes emit visible light and potentially deleterious UV radiation in the range 320–380 nm, whereas conventional tungsten filament light bulbs are safe, emitting radiations > 390 nm.

Thus photolysis is prevented by suitable packaging: low actinic amber glass bottles, cardboard outers and aluminium foil overwraps and blisters. Clear flint glass absorbs around 80% in the 290–320 nm range, whereas amber glass absorbs more than 95%. Plastic containers, by comparison, absorb only 50%.

Solid-state stability

Many of the processes of composition apply generally, particularly when the drug is in solution. However, certain important distinctions arise with the stability of drugs in the solid state, e.g. in tablets and capsules. There is limited information in the pharmaceutical literature, owing largely to the complexities of formulated systems and the difficulties in obtaining quantitative data. This paucity of data must not be interpreted to mean that this area is unimportant, especially given the popularity of tablets and capsules.

In all solid dose formulations there will be some free moisture (contributed by excipients as well as the drug), and certainly in tablets a significant percentage, typically 2% w/w, is required to facilitate good compression. This free water acts as a vector for chemical reactions between drug and excipients, and the adsorbed moisture films are saturated with drug compared to the dilute solutions encountered in injectables. The ionic equilibria are quite different and comparison is meaningless. They should not be extrapolated glibly to the solid state.

Hygroscopicity

A substance that absorbs sufficient moisture from the atmosphere to dissolve itself is deliquescent. A substance that loses water to form a lower hydrate or becomes anhydrous is termed efflorescent. These are extreme cases, and most pharmaceutical compounds are usually either impassive to the water available in the surrounding atmosphere or lose or gain water from the atmosphere, depending on the relative humidity (RH). Materials unaffected by RH are termed non-hygroscopic, whereas those in dynamic equilibrium with water in the atmosphere are hygroscopic. Ambient RH (0% poles and desert, 55% temperate and 87% tropics) can vary widely and continually depending on the weather and air temperature, and these cyclic changes lead to constant variations in the moisture content of unprotected bulk drug and excipients. The constant sinusoidal change in day and night temperatures is the major influence. For this reason pharmaceutical air conditioning is usually set below 50% RH, and very hygroscopic products, e.g. effervescents, which are particularly moisture sensitive, are stored and made below 40% RH. Tablets and capsules must be hydrophilic to facilitate wetting and the process of deaggregation and drug dissolution. As a paradox they must have limited hygroscopicity to ensure good chemical and physical stability under all reasonable climatic conditions. Good packaging will accommodate moisture challenge, e.g. glass bottles, foil blisters and dessicant. However, preformulation studies on the drug and potential excipient combinations should provide the basis for more robust formulations and a wider, more flexible and cheaper choice of pack, while still reducing significantly any hydrolytic instability due to absorbed free moisture. Pharmaceutical bulk, i.e. drug salts, should be chosen as being non-hygroscopic. As a working limit this should be <0.5% H_2O at <95% RH.

Stability assessment

The testing protocols used in preformulation to ascertain the stability of formulated products must be performed in solution and in the solid state since the same drug (salt) will be used in both an injection and a capsule, for example. These protocols are discussed briefly in Chapter 7, and a suggested scheme for preformulation samples has been shown in Table 8.11.

MICROSCOPY

The microscope has two major applications in pharmaceutical preformulation:

1. Basic crystallography, to determine crystal morphology (structure and habit), polymorphism and solvates
2. Particle size analysis.

Most pharmaceutical powders have crystals in the range 0.5–300 μm. However, the distributions are often smaller, typically 0.5–50 μm, to ensure good

blend homogeneity and rapid dissolution. These are the major reasons for particle size control.

A lamp-illuminated mono-objective microscope fitted with polarizing filters above and below the stage is more than adequate. For most preformulation work a 10 × eyepiece and a 10 × objective are ideal, although occasionally, with micronized powders and when following solid–solid and liquid–liquid transitions in polymorphism, 10 × 20 may be required.

Crystal morphology

Crystals are characterized by repetition of atoms or molecules in a regular three-dimensional structure, which is absent in glasses and some polymers. There are six crystal systems (cubic, tetragonal, orthorhombic, monoclinic, triclinic and hexagonal) which have different internal structures and spatial arrangements. Although not changing their internal structure, which occurs with polymorphism, crystals can adopt different external structures. This is known as crystal habit, of which five types are recognized:

- Tabular: moderate expansion of two parallel faces
- Platy: plates
- Prismatic: columns
- Acicular: needle-like
- Bladed: flat acicular.

These occur in all six crystal systems.

Conditions during crystallization will contribute to changes in crystal habit and may be encountered in early batches of a new drug substance until the synthetic route has been optimized. Crystal habit can be modified by:

1. Excessive supersaturation, which tends to transform a prism or isodiametric (granular) crystals to a needle shape.
2. Cooling rate and agitation, which changes habit as it changes the degree of supersaturation. Naphthalene gives thin plates (platy) if rapidly recrystallized in cold ethanol or methanol, whereas slow evaporation yields prisms.
3. The crystallizing solvent affects habit by preferential absorption on to certain faces, inhibiting their growth. Resorcinol produces needles from benzene and squat prisms from butyl acetate.
4. The addition of cosolvents or other solutes and ions which change habit by poisoning crystal growth in one or more directions. Sodium chloride is usually cubic, but urea produces an octahedral habit.

Particle size analysis

Small particles are particularly important in low-dose high-potency drug candidates, as large particle populations are necessary to ensure adequate blend homogeneity (coefficient of variation <1–2%), and for any drug whose aqueous solubility is poor (<1 mg mL^{-1}), as dissolution rate is directly proportional to surface area (inversely proportional to particle size).

There are numerous methods of particle sizing. Sieving is usually unsuitable during preformulation owing to the lack of bulk material. The simplest (but unfortunately the most tedious) method for small quantities is the microscope. The Coulter Counter (a conductivity method based on electrolyte displacement as the sample is drawn through a small hole) and laser light scattering are widely used for routine bulk analysis and research.

POWDER FLOW PROPERTIES

Of primary importance when handling a drug powder is flow. When limited amounts of drug are available this can be evaluated by measurements of bulk density and angle of repose. These are extremely useful derived parameters to assess the impact of changes in drug powder properties as new batches become available. Changes in particle size and shape are generally very apparent; an increase in crystal size or a more uniform shape will lead to a smaller angle of repose and a smaller Carr's index.

Bulk density

A simple test has been developed to evaluate the flowability of a powder by comparing the poured (fluff) density (ρ_{Bmin}) and tapped density (ρ_{Bmax}) of a powder and the rate at which it packed down. A useful empirical guide is given by Carr's compressibility index ('Compressibility' is a misnomer, as compression is not involved):

$$\text{Carr's index (\%)} = \frac{\text{Tapped} - \text{Poured density}}{\text{Tapped density}} \times 100 \quad (8.12)$$

This is a simple index that can be determined on small quantities of powder and may be interpreted as in Table 8.13.

A similar index has been defined by Hausner (1967):

$$\text{Hausner ratio} = \frac{\text{Tapped density } (\rho_{Bmax})}{\text{Poured density} (\rho_{Bmin})} \times 100 \quad (8.13)$$

Table 8.13 Carr's index as an indication of powder flow

Carr's index (%)	Type of flow
5–15	Excellent
12–16	Good
18–21	Fair to passable[a]
23–35	Poor[a]
33–38	Very poor
>40	Extremely poor

[a] May be improved by glidant, e.g. 0.2% Aerosil.

Values less than 1.25 indicate good flow (= 20% Carr), whereas greater than 1.25 indicates poor flow (= 33% Carr). Between 1.25 and 1.5, added glidant normally improves flow.

Carr's index is a one-point determination and does not always reflect the ease or speed with which the powder consolidates. Indeed, some materials have a high index (suggesting poor flow) but may consolidate rapidly. Rapid consolidation is essential for uniform filling on tablet machines, when the powder flows at ρ_{Bmin} into the die and consolidates, approaching ρ_{Bmax} at compression. An empirical linear relationship exists between the change in bulk density and the log number of taps in a jolting volumeter. Non-linearity occurs up to two taps and after 30 taps when the bed consolidates more slowly. The slope is a measure of the speed of consolidation and is useful for assessing powders or blends with similar Carr's indices and the benefit of glidants.

Angle of repose

A static heap of powder, with only gravity acting upon it, will tend to form a conical mound. One limitation exists: the angle to the horizontal cannot exceed a certain value, and this is known as the angle of repose (θ). If any particle temporarily lies outside this limiting angle, it will slide down the adjacent surface under the influence of gravity until the gravitational pull is balanced by the friction caused by interparticulate forces. Accordingly, there is an empirical relationship between θ and the ability of the powder to flow. However, the exact value for angle of repose does depend on the method of measurement. The angles of repose given in Table 8.14 may be used as a guide to flow.

A simple relationship between angle of repose, Carr's index and the expected powder flow is shown in Figure 8.6. When only small quantities of powder are available, an alternative is to determine the 'angle of spatula' by picking up a quantity of powder on a spatula and estimating the angle of the triangular section of the powder heap viewed from the end of the spatula. This is obviously crude but is useful during preformulation, when only small quantities of drug are available.

Table 8.14 Angle of repose as an indication of powder flow properties

Angle of repose (degrees)	Type of flow
<20	Excellent
20–30	Good
30–34	Passable[a]
>40	Very poor

[a] May be improved by a glidant, e.g. 0.2% Aerosil.

COMPRESSION PROPERTIES

The compression properties of most drug powders are extremely poor and necessitate the addition of compression aids. When the dose is less than 50 mg, tablets can usually be prepared by direct compression with the addition of modern direct compression bases. At higher doses the preferred method would be wet massing.

Nonetheless, information on the compression properties of the pure drug is extremely useful. Although it is true that the tableted material should be plastic, i.e. capable of permanent deformation, it should also exhibit a degree of brittleness (fragmentation). Thus if the drug dose is high and it behaves plastically, the chosen excipients should fragment, e.g. lactose, calcium phosphate. If the drug is brittle or elastic, the excipients should be plastic, i.e. microcrystalline cellulose, or plastic binders should be used in wet massing. Obviously, as the dose is reduced this becomes less important as the diluent vehicle dominates compressibility.

The compression properties (elasticity, plasticity, fragmentation and punch filming propensity) for small quantities of a new drug candidate can be established by the sequence outlined in Table 8.15. An interpretation of the results follows.

Plastic material

When materials are ductile they deform by changing shape (plastic flow). As there is no fracture, no new surfaces are generated during compression and a

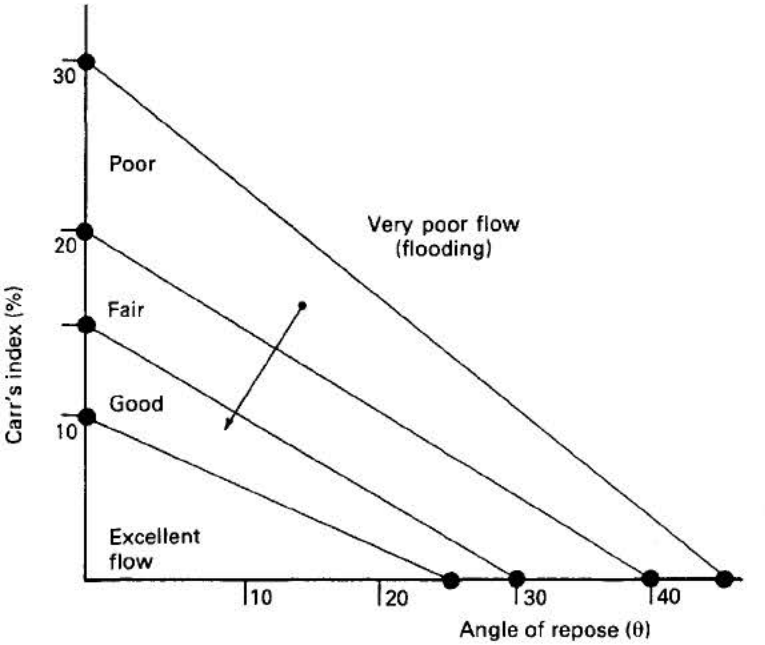

Fig. 8.6 Relationship between angle of repose, Carr's index of a powder and its flow characteristics.

Table 8.15 Scheme for the evaluation of drug compression properties

	500 mg drug + 1% magnesium stearate		
Sample code	A	B	C
Blend in a tumbler mixer for	5 min	5 min	30 min
Compress 13 mm diam. compacts in a IR hydraulic press at	75 MPa	75 MPa	75 MPa
for a dwell time of	2 s	30 s	2 s
Store tablets in a sealed container at room temperature to allow equilibration	24 h	24 h	24 h
Perform crushing strength on tablets and record load	*A* N	*B* N	*C* N

more intimate mix of magnesium stearate (as in sample C, Table 8.15) leads to poorer bonding. Because these materials bond after viscoelastic deformation, and because this is time dependent, increasing the dwell time at compression (*B*) will increase bonding strength. Thus, a material exhibiting crushing strengths in order $B > A > C$ would probably have plastic tendencies.

Fragmentation

If a material is predominantly fragmenting, neither lubricant mixing time (*C*) nor dwell time (*B*) should affect tablet strength. Thus materials which show crushing strengths which are independent of the method of manufacture outlined in Table 8.15 are likely to exhibit fragmenting properties during compression, with a high friability.

Elastic material

Some materials, e.g. paracetamol, are elastic and there is very little permanent change (either plastic flow or fragmentation) caused by compression: the material rebounds (recovers elastically) when the compression load is released. If bonding is weak the compact will self-destruct and the top will detach (capping), or the whole cylinder cracks into horizontal layers (lamination). An elastic body will give as follows:

A will cap or laminate.
B will probably maintain integrity but will be very weak.
C will cap or laminate.

Elastic materials require a particularly plastic tableting matrix or wet massing to induce plasticity.

Punch filming (sticking)

Finally, the surface of the top and bottom punches should be examined for drug adhesion. The punches can be dipped into a suitable extraction solvent, e.g. MeOH, and the drug level determined. This will probably be higher for *A* and *B* (Table 8.15), as magnesium stearate is an effective antiadherent and 30 minutes' mixing (C) should produce a monolayer and suppress adhesion more effectively.

Sticky materials can be improved by a change in salt form; by using high excipient ratios; by using abrasive inorganic excipients; by wet massing; and/or by the addition of up to 2% magnesium stearate.

Table 8.16 Suggested primary candidates as excipients for tablet and capsule formulations

Excipient	Function[a]
Lactose monohydrate	F
Dicalcium phosphate dihydate	F
Dicalcium phosphate anhydrous	F
Microcrystalline cellulose	F
Maize starch	D
Modified starch	D
Polyvinylpyrrolidone	B
Sodium starch glycollate	D
Sodium croscarmellose	D
Magnesium stearate	L
Stearic acid	L
Colloidal silica	G

[a] B, binder; D, disintegrant; F filler/diluent; G, glidant; L, lubricant.

EXCIPIENT COMPATIBILITY

The successful formulation of a stable and effective solid dosage form depends on the careful selection of the excipients that are added to facilitate administration, promote the consistent release and bioavailability of the drug and protect it from degradation.

Thermal analysis can be used to investigate and predict any physicochemical interactions between components in a formulation and can therefore be applied to the selection of suitable chemically compatible excipients. Primary excipients recommended for initial screening for tablet and capsule formulations are shown in Table 8.16.

Method

The preformulation screening of drug–excipient interactions requires 5 mg of drug, in a 50% mixture with the excipient, to maximize the likelihood of observing an interaction. Mixtures should be examined under nitrogen to eliminate oxidative and pyrrolytic effects at a standard heating rate (2, 5 or 10°C min^{-1}) on the DSC apparatus, over a temperature range which will encompass any thermal changes due to both the drug and excipient.

The melting range and any other transitions of the drug will be known from earlier investigations into purity, polymorphism and solvates. For all potential excipients (Table 8.16) it is sensible to retain representative thermograms in a reference file for comparison.

Interpretation

A scheme for interpreting DSC data from individual components and their mixtures is shown in Figure 8.7. Basically, the thermal properties of a physical mixture are the sum of the individual components, and this thermogram can be compared with those of the drug and the excipient alone. An interaction on DSC will show as changes in melting point, peak shape and area and/or the appearance of a transition. However, there is invariably some change in transition temperature and peak shape and area by virtue of mixing two components, and this is not due to any detrimental interaction. In general, provided that no new thermal events occur, no interaction can be assigned. Chemical interactions are indicated by the appearance of new peaks, or where there is gross broadening or elongation of an exo- or endothermic change. Second-order transitions produce changes in the baseline. Such observations may be indicative of the production of eutectic or solid solution-type melts. The excipient is then probably chemically reactive and incompatible with the drug, and should be avoided. Where an interaction is suspected but the thermal changes are small, the incompatibility should be confirmed by TLC.

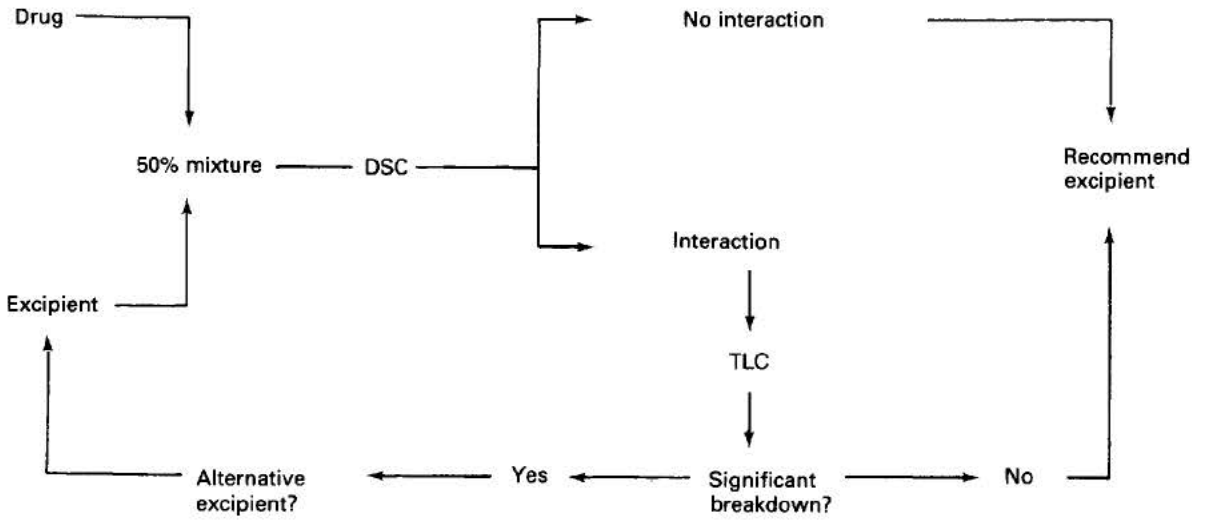

Fig. 8.7 Scheme to identify chemically compatible excipients using DSC with confirmatory TLC.

The advantages of DSC over more traditional, routine compatibility screens, typically TLC, is that no long-term storage of the mixture is required prior to evaluation, nor is any inappropriate thermal stress (other than the DSC itself, which had drug and excipient controls) required to accelerate the interactions. This, in itself, may be misleading if the mode of breakdown changes with temperature and elevated temperatures fail to reflect the degradation path occurring under normal (room temperature) storage.

Where confirmation is required by TLC, samples (50:50 mixtures of drug and excipient) should be sealed in small neutral glass test tubes and stored for either 7 days at 50°C or 14 days at 37°C.

It is important to view the results of such incompatibility testing with caution. For example, magnesium stearate is notoriously incompatible with a wide range of compounds when tested, yet because it is only used at low levels – typically 0.5–1% – such apparent incompatibility rarely produces a problem in practice in long-term storage and use.

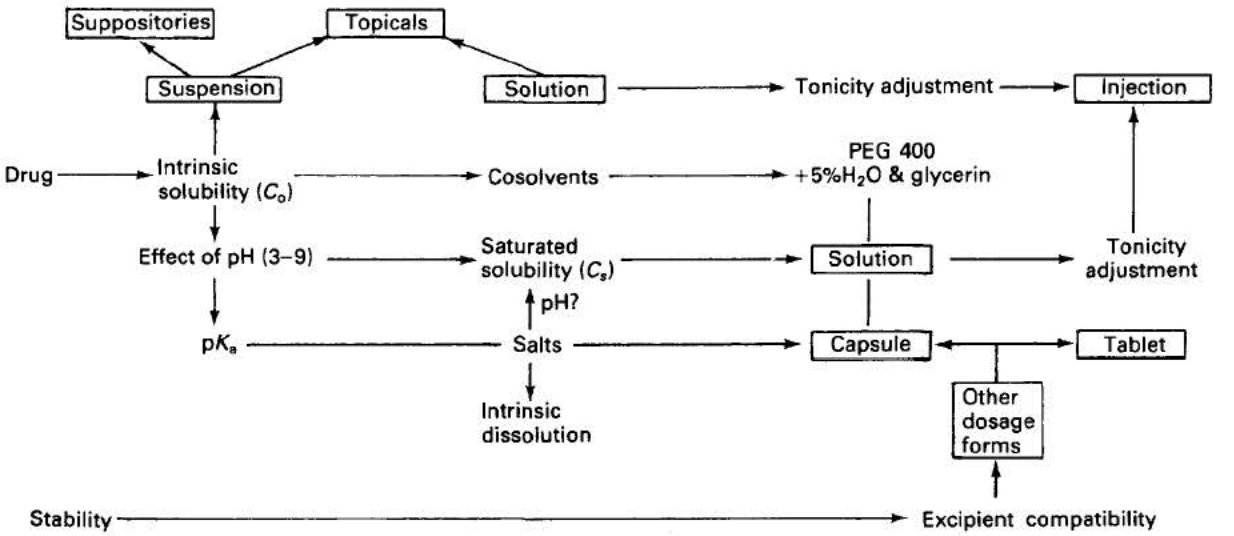

Fig. 8.8 A generic development pathway: the relationship between preformulation and formulation in dosage form development. The formulation stages are shown in boxes and the preformulation stages are unboxed.

CONCLUSIONS

Preformulation studies have a significant part to play in anticipating formulation problems and identifying logical paths in both liquid and solid dosage form technology (Fig. 8.8). The need for adequate drug solubility cannot be overemphasized. The availability of good solubility data should allow the selection of the most appropriate salt for development. Stability studies in solution will indicate the feasibility of parenteral or other liquid dosage forms, and can identify methods of stabilization. In parallel, solid-state stability by DSC, TLC and HPLC, and in the presence of tablet and capsule excipients, will indicate the most acceptable vehicles for solid dosage forms.

By comparing the physicochemical properties of each drug candidate within a therapeutic group (using C_s, pK_a, melting point, K_w^o) the preformulation scientist can assist the synthetic chemist to identify the optimum molecule, provide the biologist with suitable vehicles to elicit pharmacological response, and advise the bulk chemist about the selection and production of the best salt with appropriate particle size and morphology for subsequent processing.

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23
Suspensions and emulsions

Michael Billany

CHAPTER CONTENTS

INTRODUCTION

A coarse suspension is a dispersion of finely divided, insoluble solid particles (the disperse phase) in a fluid (the dispersion medium or continuous phase). Most pharmaceutical suspensions consist of an aqueous dispersion medium, although in some instances it may be an organic or oily liquid. A disperse phase with a mean particle diameter of up to 1 μm is usually termed a colloidal dispersion, and includes such examples as aluminium hydroxide and magnesium hydroxide suspensions. A solid in liquid dispersion, in which the particles are above colloidal size, is termed a coarse suspension.

An emulsion may be defined as two immiscible liquids, one of which is finely subdivided and uniformly distributed as droplets throughout the other. The system is stabilized by the presence of an emulsifying agent. The dispersed liquid or internal phase usually consists of globules of diameters down to 0.1 μm which are distributed within the external or continuous phase.

The physical properties of both colloidal and coarse suspensions and of emulsions are discussed in Chapter 6.

PHYSICAL PROPERTIES OF WELL-FORMULATED SUSPENSIONS AND EMULSIONS

- The product must remain sufficiently homogenous for at least the period between shaking the container and removing the required amount.
- The sediment or creaming produced on storage, if any, must be easily resuspended by moderate agitation of the container.
- The product may be required to be thickened in order to reduce the rate of settling of the particles or the rate of creaming of oil globules. The resulting viscosity must not be so high that removal of the product from the container and transfer to the site of application are difficult.
- Any suspended particles should be small and uniformly sized in order to give a smooth, elegant product, free from a gritty texture.

PHARMACEUTICAL APPLICATIONS OF SUSPENSIONS

Suspensions can be used as oral dosage forms, applied topically to the skin or mucous membrane surfaces, or given parenterally by injection.

Suspensions as oral drug delivery systems

Many people have difficulty in swallowing solid dosage forms and therefore require the drug to be dispersed in a liquid.

Some materials are required to be present in the gastrointestinal tract in a finely divided form, and their formulation as suspensions will provide the desired high surface area. Solids such as kaolin, magnesium carbonate and magnesium trisilicate, for example, are used for the adsorption of toxins, or to neutralize excess acidity. A dispersion of finely divided silica in dimethicone 1000 is used in veterinary practice for the treatment of 'frothy bloat'.

The taste of most drugs is more noticeable if it is in solution rather than in an insoluble form. Paracetamol is available both in solution as Paediatric Paracetamol Oral Solution and also as a suspension. The latter is more palatable, and therefore particularly suitable for children. For the same reason chloramphenicol mixtures can be formulated as suspensions containing the insoluble chloramphenicol palmitate.

Suspensions for topical administration

Suspensions of drugs can also be formulated for topical application (Chapter 33). They can be fluid preparations, such as Calamine Lotion, which are designed to leave a light deposit of the active agent on the skin after quick evaporation of the dispersion medium. Some suspensions, such as pastes, are semisolid in consistency and contain high concentrations of powders dispersed – usually – in a paraffin base. It may also be possible to suspend a powdered drug in an emulsion base, as in Zinc Cream.

Suspensions for parenteral use and inhalation therapy

Suspensions can also be formulated for parenteral administration in order to control the rate of absorption of the drug. By varying the size of the dispersed particles of active agent, the duration of activity can be controlled. The absorption rate of the drug into the bloodstream will then depend simply on its rate of dissolution. If the drug is suspended in a fixed oil such as arachis or sesame, the product will remain after injection in the form of an oil globule, thereby presenting to the tissue fluid a small surface area from which the partitioning of drug can occur. The release of drug suspended in an aqueous vehicle will be faster, as some diffusion of the product will occur along muscle fibres and become miscible with tissue fluid. This will present a larger surface area from which the drug can be released.

Vaccines for the induction of immunity are often formulated as dispersions of killed microorganisms, as in Cholera Vaccine, or of the constituent toxoids adsorbed on to a substrate of aluminium hydroxide or phosphate, as in Adsorbed Diphtheria and Tetanus Vaccine. Thus a prolonged antigenic stimulus is provided, resulting in a high antibody titre.

Some X-ray contrast media are also formulated in this way. Barium sulphate, for the examination of the alimentary tract, is available as a suspension for either oral or rectal administration, and propyliodone is dispersed in either water or arachis oil for examination of the bronchial tract.

The adsorptive properties of fine powders are also used in the formulation of some inhalations. The volatile components of menthol and eucalyptus oil would be lost from solution very rapidly during use, whereas a more prolonged release is obtained if the two active agents are adsorbed on to light magnesium carbonate prior to the preparation of a suspension.

Chapter 31 describes some aspects of the formulation of aerosols, many of which are also available as suspensions of the active agent in a mixture of propellants.

Solubility and stability considerations

If the drug is insoluble or poorly soluble in a suitable solvent, then formulation as a suspension is usually required. Some eye drops, notably Hydrocortisone Acetate and Neomycin Eye Drops, are formulated as suspensions because of the poor solubility of hydrocortisone in a suitable solvent.

The degradation of a drug in the presence of water may also preclude its use as an aqueous solution. In this case it may be possible to synthesize an insoluble derivative that can then be formulated as a suspension. For example, oxytetracycline hydrochloride is used in solid dosage forms, but in aqueous solution would rapidly hydrolyse. A stable liquid dosage form has been made by suspending the insoluble calcium salt in a suitable aqueous vehicle.

Prolonged contact between the solid drug particles and the dispersion medium can be considerably reduced by preparing the suspension immediately prior to issue to the patient. Amoxicillin, for example, is provided by the manufacturer as the trihydrate salt mixed with the other powdered or granulated ingredients. The pharmacist then makes the product up to volume with water immediately before issue to the patient, allocating a shelf-life of 14 days at a temperature at or below 25°C.

A drug that degrades in the presence of water may alternatively be suspended in a non-aqueous vehicle. Fractionated coconut oil is used as the vehicle for some formulations of antibiotics for oral use, and in some countries tetracycline hydrochloride is dispersed in a similar base for ophthalmic use.

FORMULATION OF SUSPENSIONS

Particle size control

It is first necessary to ensure that the drug to be suspended is of a fine particle size prior to formulation. This is to ensure a slow rate of sedimentation of the suspended particles. Large particles, if greater than about 5 μm diameter, will also impart a gritty texture to the product, and may cause irritation if injected or instilled into the eyes. The ease of administration of a parenteral suspension may depend upon particle size and shape, and it is quite possible to block a hypodermic needle with particles over about 25 μm diameter, particularly if they are acicular in shape rather than isodiametric. A particular particle size range may also be chosen in order to control the rate of dissolution of the drug and hence its bioavailability.

Even though the particle size of a drug may be small when the suspension is first manufactured, there is always a degree of crystal growth that occurs on storage, particularly if temperature fluctuations occur. This is because the solubility of the drug may increase as the temperature rises, but on cooling, the drug will crystallize out. This is a particular problem with slightly soluble drugs such as paracetamol.

If the drug is polydispersed, then the very small crystals of less than 1 μm diameter will exhibit a greater solubility than the larger ones. Over a period of time the small crystals will become even smaller, whereas the diameters of the larger particles will increase. It is therefore advantageous to use a suspended drug of a narrow size range. The inclusion of surface-active agents or polymeric colloids, which adsorb on to the surface of each particle, may also help to prevent crystal growth.

Different polymorphic forms of a drug may exhibit different solubilities, the metastable state being the most soluble. Conversion of the metastable form, in solution, to the less soluble stable state, and its subsequent precipitation, will lead to changes in particle size.

The use of wetting agents

Some insoluble solids may be easily wetted by water and will disperse readily throughout the aqueous phase with only minimal agitation. Most, however, will exhibit varying degrees of hydrophobicity and will not be easily wetted. Some particles will form large porous clumps within the liquid, whereas others remain on the surface and become attached to the upper part of the container. The foam produced on shaking will be slow to subside because of the stabilizing effect of the small particles at the liquid/air interface.

To ensure adequate wetting, the interfacial tension between the solid and the liquid must be reduced so that the adsorbed air is displaced from the solid surfaces by the liquid. The particles will then disperse readily throughout the liquid, particularly if an intense shearing action is used during mixing. If a series of suspensions is prepared, each containing one of a range of concentrations of wetting agent, then the concentration to choose will be the lowest that provides adequate wetting.

The following is a discussion of the most widely used wetting agents for pharmaceutical products.

Surface-active agents

Figure 6.15 shows that surfactants possessing an HLB value between about 7 and 9 would be suitable for use as wetting agents. The hydrocarbon chains would be adsorbed by the hydrophobic particle surfaces, whereas the polar groups project into the aqueous medium and become hydrated. Wetting of the solid occurs as a result of a fall both in interfacial tension between the solid and the liquid and, to a lesser extent, between the liquid and air.

Most surfactants are used at concentrations of up to about 0.1% as wetting agents and include, for oral use, the polysorbates (Tweens) and sorbitan esters (Spans). For external application, sodium lauryl sulphate, sodium dioctylsulphosuccinate and quillaia extract can also be used.

The choice of surfactant for parenteral administration is obviously more limited, the main ones used

being the polysorbates, some of the poloxamers (polyoxyethylene/polyoxypropylene copolymers) and lecithin.

Disadvantages in the use of this type of wetting agent include excessive foaming and the possible formation of a deflocculated system, which may not be required.

Hydrophilic colloids

These materials include acacia, bentonite, tragacanth, alginates, xanthan gum and cellulose derivatives, and will behave as protective colloids by coating the solid hydrophobic particles with a multimolecular layer. This will impart a hydrophilic character to the solid and so promote wetting. These materials are also used as suspending agents and may, like surfactants, produce a deflocculated system, particularly if used at low concentrations.

Solvents

Materials such as alcohol, glycerol and glycols, which are water miscible, will reduce the liquid/air interfacial tension. The solvent will penetrate the loose agglomerates of powder displacing the air from the pores of the individual particles, so enabling wetting to occur by the dispersion medium.

Flocculated and deflocculated systems

Having incorporated a suitable wetting agent, it is then necessary to determine whether the suspension is flocculated or deflocculated and to decide which state is preferable. Whether or not a suspension is flocculated or deflocculated depends on the relative magnitudes of the forces of repulsion and attraction between the particles. The effects of these particle–particle interactions have been adequately covered in Chapter 6.

In a deflocculated system the dispersed particles remain as discrete units and, because the rate of sedimentation depends on the size of each unit, settling will be slow. The supernatant of a deflocculated system will continue to remain cloudy for an appreciable time after shaking, due to the very slow settling rate of the smallest particles in the product, even after the larger ones have sedimented. The repulsive forces between individual particles allow them to slip past each other as they sediment. The slow rate of settling prevents the entrapment of liquid within the sediment, which thus becomes compacted and can be very difficult to redisperse. This phenomenon is also called caking or claying, and is the most serious of all the physical stability problems encountered in suspension formulation.

The aggregation of particles in a flocculated system will lead to a much more rapid rate of sedimentation or subsidence because each unit is composed of many individual particles and is therefore larger. The rate of settling will also depend on the porosity of the aggregate, because if it is porous the dispersion medium can flow through, as well as around, each aggregate or floccule as it sediments.

The nature of the sediment of a flocculated system is also quite different from that of a deflocculated one. The structure of each aggregate is retained after sedimentation, thus entrapping a large amount of the liquid phase. As explained in Chapter 6, aggregation in the primary minimum will produce compact floccules, whereas a secondary minimum effect will produce loose floccules of higher porosity. Whichever occurs, the volume of the final sediment will still be large and will easily be redispersed by moderate agitation.

In a flocculated system the supernatant quickly becomes clear, as the large flocs that settle rapidly are composed of particles of all sizes. Figure 23.1 illus-

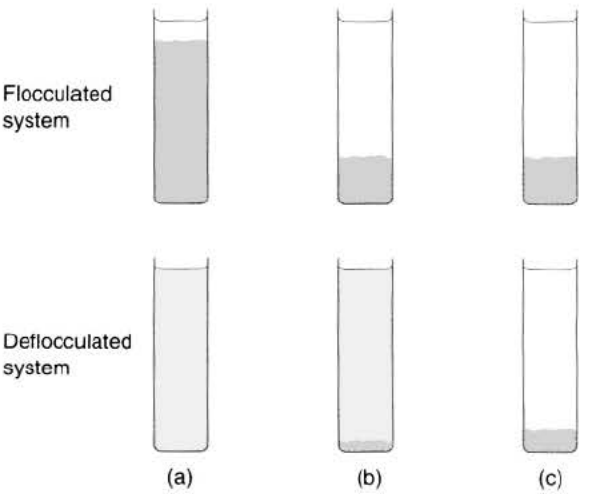


Fig. 23.1 The sedimentation behaviour of flocculated and deflocculated suspensions. Within a few minutes of manufacture (a) there is no apparent change within the deflocculated system compared to its initial appearance. Even after several hours (b) there is still little obvious change, except that the concentration of solids in the lower layers has increased at the expense of the upper layers owing to slow particle sedimentation. There is a small amount of a compact sediment. After prolonged storage (c), depending on the physical stability of the system, the supernatant has cleared, leaving a compact sediment. In the flocculated system at (a) there is some clear supernatant with a distinct boundary between it and the sediment. At (b) there is a larger volume of clear supernatant with a relatively large volume of a porous sediment, which does not change further even after prolonged storage (c).

trates the appearance of both flocculated and deflocculated suspensions at given times after shaking.

In summary, deflocculated systems have the advantage of a slow sedimentation rate, thereby enabling a uniform dose to be taken from the container, but when settling does occur the sediment is compacted and difficult to redisperse. Flocculated systems form loose sediments which are easily redispersible, but the sedimentation rate is fast and there is a danger of an inaccurate dose being administered; also, the product will look inelegant.

Controlled flocculation

A deflocculated system with a sufficiently high viscosity to prevent sedimentation would be an ideal formulation. It cannot be guaranteed, however, that the system would remain homogenous during the entire shelf-life of the product. Usually a compromise is reached in which the suspension is partially flocculated to enable adequate redispersion if necessary, and viscosity is controlled so that the sedimentation rate is at a minimum.

The next stage of the formulation process, after the addition of the wetting agent, is to ensure that the product exhibits the correct degree of flocculation. Underflocculation will give those undesirable properties that are associated with deflocculated systems. An overflocculated product will look inelegant and, to minimize settling, the viscosity of the product may have to be so high that any necessary redispersion would be difficult.

Controlled flocculation is usually achieved by a combination of particle size control, the use of electrolytes to control zeta potential, and the addition of polymers to enable crosslinking to occur between particles. Some polymers have the advantage of becoming ionized in an aqueous solution, and can therefore act both electrostatically and sterically. These materials are also termed polyelectrolytes.

Flocculating agents

In many cases, after the incorporation of a non-ionic wetting agent a suspension will be found to be deflocculated, either because of the reduction in solid/liquid interfacial tension, or because of the hydrated hydrophilic layer around each particle forming a mechanical barrier to aggregation. The use of an ionic surfactant to wet the solid could produce either a flocculated or a deflocculated system, depending on any charge already present on the particles. If particles are of opposite charge to that of the surfactant then neutralization will occur. If a high charge density is imparted to the suspended particles then deflocculation will be the result.

If it is necessary for the suspension to be converted from a deflocculated to a partially flocculated state, this may be achieved by the addition of electrolytes, surfactants and/or hydrophilic polymers.

Electrolytes The addition of an inorganic electrolyte to an aqueous suspension will alter the zeta potential of the dispersed particles and, if this value is lowered sufficiently, flocculation may occur.

The Schultz–Hardy rule shows that the ability of an electrolyte to flocculate hydrophobic particles depends on the valency of its counter-ions. Although they are more efficient, trivalent ions are less widely used than mono- or divalent electrolytes because they are generally more toxic. If hydrophilic polymers, which are usually negatively charged, are included in the formulation they may be precipitated by the presence of trivalent ions.

The most widely used electrolytes include the sodium salts of acetates, phosphates and citrates, and the concentration chosen will be that which produces the desired degree of flocculation. Care must be taken not to add excessive electrolyte or charge reversal may occur on each particle, so forming, once again, a deflocculated system.

Surfactants Ionic surface-active agents may also cause flocculation by neutralizing the charge on each particle, thus resulting in a deflocculated system. Non-ionic surfactants will, of course, have a negligible effect on the charge density of a particle but may, because of their linear configurations, adsorb on to more than one particle, thereby forming a loose flocculated structure.

Polymeric flocculating agents Starch, alginates, cellulose derivatives, tragacanth, carbomers and silicates are examples of polymers that can be used to control flocculation. Their linear branched-chain molecules form a gel-like network within the system and become adsorbed on to the surfaces of the dispersed particles, thus holding them in a flocculated state. Although some settling can occur, the sedimentation volume is large, and usually remains so for a considerable period.

Care must be taken to ensure that, during manufacture, blending is not excessive as this may inhibit the crosslinking between adjacent particles and result in the adsorption of each molecule of polymer on to one particle only. If this should occur then a deflocculated system may result, because the formation of the hydrophilic barrier around each particle will inhibit aggregation. A high concentration of polymer may have a similar effect if the whole surface of each particle is coated. It is essential that

areas on each suspended particle remain free from adsorbate, so that crosslinking can recur after the product is sheared. Further details of the use of polymers can be found in the next section.

Rheology of suspensions

An ideal pharmaceutical suspension would exhibit a high apparent viscosity at low rates of shear so that, on storage, the suspended particles would either settle very slowly or, preferably, remain permanently suspended. At higher rates of shear, such as those caused by moderate shaking of the product, the apparent viscosity should fall sufficiently for the product to be poured easily from its container. The product, if for external use, should then spread easily without excessive dragging, but should not be so fluid that it runs off the skin surface. If intended for injection, the product should pass easily through a hypodermic needle with only moderate pressure applied to the syringe plunger. It would then be important for the initial high apparent viscosity to be reformed after a short time to maintain adequate physical stability.

A flocculated system partly fulfils these criteria. In such a system pseudoplastic or plastic behaviour (see Chapter 4) is exhibited as the structure progressively breaks down under shear. The product then shows the time-dependent reversibility of this loss of structure, which is termed thixotropy.

A deflocculated system, however, would exhibit newtonian behaviour owing to the absence of such structures and may even, if high concentrations of disperse phase are present, exhibit dilatancy.

Although a flocculated system may exhibit some thixotropy and plasticity, unless a high concentration of disperse phase is present it may not be sufficient to prevent rapid settling, particularly if a surfactant or an electrolyte is present as a flocculating agent. In these cases suspending agents may be used to increase the apparent viscosity of the system.

Suitable materials are the hydrophilic polymers discussed above. These exert their effect by entrapping the solid dispersed particles within their gel-like network, so preventing sedimentation. At low concentrations many suspending agents can be used to control flocculation, and it must be realized that if large quantities are to be used to enhance viscosity the degree of flocculation may also be altered.

Viscosity modifiers

The following materials are those most widely used for the modification of suspension viscosity.

Polysaccharides

Acacia This natural material is often used as a suspending agent for extemporaneously prepared suspensions. Acacia is not a good thickening agent and its value as a suspending agent is largely due to its action as a protective colloid. It is therefore useful for preparations containing tinctures of resinous materials that precipitate on addition to water. It is essential to ensure that any precipitated resin is well coated by the protective colloid before any electrolyte (which should be well diluted) is added. Acacia is not very effective for dense powders, and for these it is often combined with other thickeners such as tragacanth, starch and sucrose in compound tragacanth powder.

Unfortunately, acacia mucilage becomes acidic on storage as a result of enzyme activity, and it also contains an oxidase enzyme which may cause deterioration of active agents that are susceptible to oxidation. This enzyme can, however, be inactivated by heat.

Because of the stickiness of acacia it is rarely used in preparations for external use.

Tragacanth This product will form viscous aqueous solutions. Its thixotropic and pseudoplastic properties make it a better thickening agent than acacia and it can be used both for internal and external products. Like acacia it is mainly, though not exclusively, used for the extemporaneous preparation of suspensions with a short shelf-life.

Tragacanth is stable over a pH range of 4–7.5 but takes several days to hydrate fully after dispersion in water. The maximum viscosity of its dispersions is not, therefore, achieved until after this time, and can also be affected by heating. There are several grades of this material and only the best quality is suitable for use as a pharmaceutical suspending agent.

Alginates Alginic acid, a polymer of D-mannuronic acid, is prepared from kelp, and its salts have suspending properties similar to those of tragacanth. Alginate mucilages must not be heated above 60°C as depolymerization occurs, with a consequent loss in viscosity. They are most viscous immediately after preparation, after which there is a fall to a fairly constant value after about 24 hours. Alginates exhibit a maximum viscosity over a pH range of 5–9, and at low pH the acid is precipitated. Sodium alginate (Manucol) is the most widely used material in this class but it is, of course, anionic and will be incompatible with cationic materials and with heavy metals. The addition of calcium chloride to a sodium alginate dispersion will produce calcium alginate, which has a much higher viscosity. Several different viscosity grades are commercially available.

Starch Starch is rarely used on its own as a suspending agent but is one of the constituents of compound tragacanth powder, and it can also be used with carmellose sodium. Sodium starch glycollate (Explotab, Primojel), a derivative of potato starch, has also been evaluated for its use in the extemporaneous preparation of suspensions.

Xanthan gum (Keltrol) This is an anionic heteropolysaccharide produced by the action of *Xanthomonas campestris* on corn sugars. It is very soluble in cold water and is one of the most widely used thickening agents for the extemporaneous preparation of suspensions for oral use. It is used in concentrations up to about 2% and is stable over a wide pH range.

Water-soluble celluloses

Several cellulose derivatives are available that will disperse in water to produce viscous colloidal solutions suitable for use as suspending agents.

Methylcellulose (Celacol, Methocel) This is a semi-synthetic polysaccharide of the general formula:

$$[C_6H_7O_2(OH_2)OCH_3]_n$$

and is produced by the methylation of cellulose. Several grades are available, depending on their degree of methylation and on the chain length. The longer the chain, the more viscous is its solution. For example, a 2% solution of methylcellulose 20 exhibits an apparent viscosity of 20 millipascal seconds (mPa s) and methylcellulose 4500 has value of 4500 mPa s at 2% concentration. Because these products are more soluble in cold water than in hot, they are often dispersed in warm water and then, on cooling with constant stirring, a clear or opalescent viscous solution is produced. Methylcelluloses are non-ionic and therefore stable over a pH range of 3–11, and are compatible with many ionic additives. When these dispersions are heated, the methylcellulose molecules become progressively dehydrated and eventually gel at about 50°C; on cooling the original form is regained.

Hydroxyethylcellulose (Natrosol) This compound has hydroxyethyl instead of methyl groups attached to the cellulose chain and is also available in different viscosity grades. It has the advantage of being soluble in both hot and cold water and will not gel on heating. Otherwise it exhibits the same properties as methylcellulose.

Carmellose sodium (sodium carboxymethylcellulose) This material can be represented by:

$$[C_6H_{10-x}O_5(CH_2COONa)_x]_n$$

where x represents the degree of substitution, usually about 0.7, which in turn affects its solubility. The viscosity of its solution depends on the value of n, which represents the degree of polymerization. The numerical suffix gives an indication of the viscosity of a 2% solution. For example sodium carboxymethylcellulose 50 at a concentration of 2% will have a viscosity of 50 mPa s. This material produces clear solutions in both hot and cold water, which are stable over a pH range of about 5–10. Being anionic, this material is incompatible with polyvalent cations and the acid will be precipitated at low pHs. Heat sterilization of either the powder or its mucilage will reduce the viscosity, and this must be taken into account during formulation. It is widely used at concentrations of up to 1% in products for oral, parenteral or external use.

Microcrystalline cellulose This material consists of crystals of colloidal dimensions which disperse readily in water (but are not soluble) to produce thixotropic gels. It is a widely used suspending agent and the rheological properties of its dispersions can often be improved by the incorporation of additional hydrocolloid, in particular carboxymethylcellulose, methylcellulose and hydroxypropylmethylcellulose. These will aid dispersion and also stabilize the product against the flocculating effects of added electrolyte.

Hydrated silicates

There are three important materials within this classification, namely bentonite, magnesium aluminium silicate and hectorite, and they belong to a group called the montmorillonite clays. They hydrate readily, absorbing up to 12 times their weight of water, particularly at elevated temperatures. The gels formed are thixotropic and therefore have useful suspending properties. As with most naturally occurring materials they may be contaminated with spores, and this must be borne in mind when considering a sterilization process and choosing a preservative system.

Bentonite This has the general formula:

$$Al_2O_3.4SiO_2.H_2O$$

It is used at concentrations of up to 2 or 3% in preparations for external use, such as calamine lotion. As this product may contain pathogenic spores it should be sterilized before use.

Magnesium aluminium silicate (Veegum) Also known as attapulgite, this is available as insoluble flakes that disperse and swell readily in water by absorbing the aqueous phase into its crystal lattice. Several grades are available, differing in their particle size, their acid demand and the viscosity of their

dispersions. They can be used both internally and externally at concentrations of up to about 5%, and are stable over a pH range of 3.5–11. Veegum/water dispersions will exhibit thixotropy and plasticity with a high yield value, but the presence of salts can alter these rheological properties because of the flocculating effect of their positively charged counter-ions. Some grades, however, have a higher resistance to flocculation than others.

This material is often combined with organic thickening agents such as sodium carboxymethylcellulose or xanthan gum to improve yield values and degree of thixotropy, and to control flocculation (Ciullo 1981).

Hectorite This material is similar to bentonite and can be used at concentrations of 1–2% for external use. It is also possible to obtain synthetic hectorites (Laponite) that do not exhibit the batch variability or level of microbial contamination associated with natural products, and which can also be used internally.

As with other clays it is often advantageous to include an organic gum to modify its rheological properties.

Carbomers (carboxypolymethylene)

This material is a totally synthetic copolymer of acrylic acid and allyl sucrose. It is used at concentrations of up to 0.5%, mainly for external application, although some grades can be taken internally. When dispersed in water it forms acidic, low-viscosity solutions which, when adjusted to a pH of between 6 and 11, become highly viscous.

Colloidal silicon dioxide (Aerosil)

When dispersed in water this finely divided product will aggregate, forming a three-dimensional network. It can be used at concentrations of up to 4% for external use, but has also been used for thickening non-aqueous suspensions.

TYPES OF EMULSION

Pharmaceutical emulsions usually consist of a mixture of an aqueous phase with various oils and/or waxes. If the oil droplets are dispersed throughout the aqueous phase the emulsion is termed oil-in-water (o/w). A system in which the water is dispersed throughout the oil is a water-in-oil (w/o) emulsion. It is also possible to form multiple emulsions. For example, many small water droplets can be enclosed within larger oil droplets, which are themselves then dispersed in water. This gives a water-in-oil-in-water (w/o/w) emulsion. The alternative o/w/o emulsion is also possible.

If the dispersed globules are of colloidal dimensions (1 nm to 1 μm diameter) the preparation, which is quite often transparent or translucent, is called a microemulsion. This type has similar properties to a micellar system and will therefore exhibit the properties of hydrophobic colloids. As the size of the dispersed droplets increases more of the characteristics of coarse dispersions will be exhibited (see Chapter 6).

Tests for identification of emulsion type

Several simple methods are available for distinguishing between o/w and w/o emulsions (Table 23.1). The most common of these involve:

- miscibility tests with oil or water. The emulsion will only be miscible with liquids that are miscible with its continuous phase;
- conductivity measurements. Systems with aqueous continuous phases will readily conduct electricity, whereas systems with oily continuous phases will not;
- staining tests. Water-soluble and oil-soluble dyes are used, one of which will dissolve in, and colour the continuous phase.

FORMULATION OF EMULSIONS

Because of the very wide range of emulsifying agents available, considerable experience is required to choose the best emulgent system for a particular product. The final choice will depend to a large extent on the properties and use of the final product and the other materials required to be present.

Choice of emulsion type

The decision as to whether an o/w or a w/o emulsion is to be formulated will eliminate many unsuitable emulsifying systems.

Fats or oils for oral administration, either as medicaments in their own right or as vehicles for oil-soluble drugs, are invariably formulated as oil-in-water emulsions. In this form they are pleasant to take, and the inclusion of a suitable flavour in the aqueous phase will mask any unpleasant taste.

Table 23.1 Tests for identification of emulsion type

Oil-in-water emulsions	Water-in-oil emulsions
Miscibility tests	
Are miscible with water but immiscible with oil	Are miscible with oil but not with water
Staining tests by incorporation of an oil-soluble dye	
Macroscopic examination	
Paler colour than a w/o emulsion	More intense colouration than with an o/w emulsion
Microscopic examination	
Coloured globules on a colourless background	Colourless globules against a coloured background
Conductivity tests	
Water, being the continuous phase, will conduct electricity throughout the system. Two electrodes, when placed in such a preparation with a battery and suitable light source connected in series, will cause the lamp to glow	A preparation in which oil is the continuous phase will not conduct electricity. The lamp will not glow, or will only flicker spasmodically

Emulsions for intravenous administration must also be of the o/w type, although intramuscular injections can also be formulated as w/o products if a water-soluble drug is required for depot therapy.

Emulsions are most widely used for external application. Semisolid emulsions are termed creams and more fluid preparations are called either lotions or, if intended for massage into the skin, liniments. Both o/w and w/o types are available. The former is used for the topical application of water-soluble drugs, mainly for local effect. They do not have the greasy texture associated with oily bases and are therefore pleasant to use and easily washed from skin surfaces.

Water-in-oil emulsions will have an occlusive effect by hydration of the upper layers of the stratum corneum and the inhibition of evaporation of eccrine secretions. This, in turn, may influence the absorption rates of drugs from these preparations.

This type of emulsion is also useful for cleansing the skin of oil-soluble dirt, although its greasy texture is not always cosmetically acceptable. Oil-in-water emulsions are less efficient as cleansers but are usually more acceptable to the consumer, particularly for use on the hands. Similarly, moisturising creams, designed to prevent moisture loss from the skin and thus inhibit drying of the stratum corneum, are more efficient if formulated as w/o emulsions, which produce a coherent, water-repellent film.

Choice of oil phase

In many instances the oil phase of an emulsion is the active agent, and therefore its concentration in the product is predetermined. Liquid paraffin, castor oil, cod liver oil and arachis oil are all examples of medicaments which are formulated as emulsions for oral administration. Cottonseed oil, soya bean oil and safflower oil are used for their high calorific value in emulsions for intravenous feeding, and examples of externally applied oils that are formulated as emulsions include turpentine oil and benzyl benzoate.

Many emulsions for external use contain oils that are present as carriers for the active agent. It must be realized that the type of oil used may also have an effect both on the viscosity of the product and on the transport of the drug into the skin (see Chapter 33). One of the most widely used oils for this type of preparation is liquid paraffin. This is one of a series of hydrocarbons, which also includes hard paraffin, soft paraffin and light liquid paraffin. They can be used individually or in combination with each other to control emulsion consistency. This will ensure that the product can be spread easily but will be sufficiently viscous to form a coherent film over the skin. The film-forming capabilities of the emulsion can be further modified by the inclusion of various waxes, such as beeswax, carnauba wax or higher fatty alcohols. Continuous films can therefore be formed that are sufficiently tough and flexible to prevent contact between the skin and aqueous-based irritants. These preparations are called barrier creams, and many are of the w/o variety. The inclusion of silicone oils, such as dimethicone at 10–20%, which have exceptional water-repellent properties, may also permit the formulation of o/w products that are equally effective.

A variety of fixed oils of vegetable origin are also available, the most widely used being arachis, sesame, cottonseed and maize. Those expressed from seeds or fruits are often protein rich and contain useful vitamins and minerals. They are often, therefore, formulated for oral use as emulsions. Because of their lack of toxicity they can be used both internally and externally as vehicles for other materials.

Emulsion consistency

The texture or feel of a product intended for external use must also be considered. A w/o preparation will have a greasy texture and often exhibits a higher apparent viscosity than o/w emulsions. This fact is often used to convey a feeling of richness to many cosmetic formulations. Oil-in-water emulsions will, however, feel less greasy or sticky on application to the skin, will be absorbed more readily because of their lower oil content, and can be more easily washed from the skin surface.

Ideally emulsions should exhibit the rheological properties of plasticity/pseudoplasticity and thixotropy (see Chapter 4). A high apparent viscosity at the very low rates of shear caused by movement of dispersed phase globules is necessary in order to retard this movement and maintain a physically stable emulsion. It is important, however, that these products should flow freely when shaken, poured from the container or injected through a hypodermic needle. Therefore, at these high rates of shear, a lower apparent viscosity is required. This change in apparent viscosity must be reversible after a suitable time delay so as to retard creaming and coalescence.

For an externally applied product a wide range of emulsion consistencies can be tolerated. Low-viscosity lotions and liniments can be formulated that are dispensed from a flexible plastic container via a nozzle on to the skin. Only light shearing is then required to spread this type of product over the skin surface. This is particularly advantageous for painful or inflamed skin conditions.

The main disadvantage with low-viscosity emulsions is their tendency to cream easily, especially if formulated with a low oil concentration. It is rarely possible to formulate low-viscosity w/o products because of the consistency of the oil phase.

Emulsions of high apparent viscosity for external use are termed creams and are of a semisolid consistency. They are usually packed into collapsible plastic or aluminium tubes, although large volumes or very high-viscosity products are often packed into glass or plastic jars.

It is important not to ignore the patient/consumer acceptability of topically applied preparations, particularly in a competitive market.

There are several methods by which the rheological properties of an emulsion can be controlled.

Volume concentration of the dispersed phase

As discussed in Chapters 4 and 6, Einstein developed an equation relating the viscosity of a suspension to the volume fraction of the particles in that suspension. A qualitative application of this equation to the behaviour of emulsions shows that the viscosity of the product as a whole would be higher than the viscosity of the continuous phase on its own. So, as the concentration of dispersed phase increases, so does the apparent viscosity of the product.

Care must be taken to ensure that the dispersed phase concentration does not increase above about 60% of the total, as phase inversion may occur.

Particle size of the dispersed phase

It is possible, under certain conditions, to increase the apparent viscosity of an emulsion by a reduction in mean globule diameter. This can be achieved by homogenization. There are two postulated mechanisms for this occurrence:

1. A smaller mean globule size can cause increased flocculation. In a flocculated system a significant part of the continuous phase is trapped within aggregates of droplets, thus effectively increasing the apparent disperse phase concentration. Emulsions consisting of polydispersed droplets will tend to exhibit a lower viscosity than a monodispersed system, due to differences in electrical double-layer size and thus in the energy of interaction curves. These variations in interaction between globules during shear may be reflected in their flow behaviour,
2. If a hydrophilic colloid is used to stabilize the emulsion it will form a multimolecular film round the dispersed globules. A reduction in mean globule size will increase the total surface area, and therefore more colloid will be adsorbed on to the droplet surface. This will effectively increase the volume concentration of the dispersed phase.

The particle size of the dispersed phase is therefore controlled mainly by the method and conditions of manufacture of the emulsion, and by the type of emulgent used and its concentration.

Viscosity of the continuous phase

It has been well documented that a direct relationship exists between the viscosity of an emulsion and the viscosity of its continuous phase. Syrup and glycerol, which are used in oral emulsions as sweetening agents, will increase the viscosity of the continuous phase. Their main disadvantage is in increasing the density difference between the two phases, and thus possibly accelerating creaming.

Hydrocolloids, when used as emulsifying agents in o/w emulsions, will stabilize them not only by the formation of multimolecular layers around the dispersed globules, but also by increasing the continuous phase viscosity. They do not have the disadvantage of altering the density of this phase. If oil is the continuous phase, then the inclusion of soft or hard paraffin or certain waxes will increase its viscosity.

Viscosity of the dispersed phase

For most practical applications it is doubtful whether this factor would have any significant effect on total emulsion viscosity. It is possible, however, that a less viscous dispersed phase would, during shear, be deformed to a greater extent than a more viscous phase, and thus the total interfacial area would increase slightly. This may affect double-layer interactions and hence the viscosity of the emulsion.

Nature and concentration of the emulsifying system

It has already been shown that hydrophilic colloids, as well as forming multimolecular films at the oil/water interface, will also increase the viscosity of the continuous phase of an o/w emulsion. Obviously, as the concentration of this type of emulgent increases so will the viscosity of the product.

Surface-active agents forming condensed monomolecular films will, by the nature of their chemical structure, influence the degree of flocculation in a similar way, by forming linkages between adjacent globules and creating a gel-like structure. A flocculated system will exhibit a greater apparent viscosity than its deflocculated counterpart and will depend on surfactant concentration.

Choice of emulsifying agent

Toxicity and irritancy considerations

The choice of emulgent to be used will depend not only on its emulsifying ability, but also on its route of administration and, consequently, on its toxicity. Although there is no approved list of emulsifying agents for use in pharmaceutical products there is an approved list of emulsifiers as food additives for use in the European Union. It can be assumed that emulsifiers contained in this list would be suitable for internally used pharmaceutical emulsions. The regulations mainly include naturally occurring materials and their semisynthetic derivatives, such as the polysaccharides, as well as glycerol esters, cellulose ethers, sorbitan esters and polysorbates.

It will be noted that most of these are non-ionic, having a tendency to be less irritant and less toxic than their anionic, and particularly their cationic counterparts. The concentrations of ionic emulsifying agents necessary for emulsification will be irritant to the gastrointestinal tract and have a laxative effect, and should not be used for oral emulsions. Cationic surfactants in general are toxic even at lower concentrations. The emulgent cetrimide is limited to externally used preparations, where its antiseptic properties are of use.

Some emulgents, such as the anionic alkali soaps, often have a high pH and are thus unsuitable for application to broken skin. Even on normal intact skin with a pH of 5.5, the application of such alkaline materials can cause irritation. Some emulsifiers, in particular, wool fat can cause sensitization reactions in susceptible people.

When choosing an emulgent for parenteral use it must be realized that only certain types of non-ionic material are suitable. These include lecithin, polysorbate 80, methylcellulose, gelatin and serum albumin.

Formulation by the HLB method

It has already been shown that physically stable emulsions are best achieved by the presence of a condensed layer of emulgent at the oil/water interface, and that the complex interfacial films formed by a blend of an oil-soluble emulsifying agent with a water-soluble one produces the most satisfactory emulsions.

A useful method has been devised for calculating the relative quantities of these emulgents necessary to produce the most physically stable emulsion for a particular oil/water combination. This is called the hydrophile–lipophile balance (HLB) method. Although originally applied to non-ionic surface-active agents, its use has been extended to ionic emulgents. Each surfactant is allocated an HLB number representing the relative proportions of the lipophilic and hydrophilic parts of the molecule. High numbers (up to a theoretical maximum of 20) therefore indicate a surfactant exhibiting mainly hydrophilic or polar properties, whereas low numbers represent lipophilic or non-polar characteristics. Table 23.2 gives HLB values for some commonly used emulsifying agents. The concept of HLB values is discussed more fully in Chapter 6.

Each type of oil used will require an emulgent of a particular HLB number in order to ensure a stable

Table 23.2 HLB values for some pharmaceutical surfactants

Sorbitan trioleate (Span 85)	1.8
Oleic acid	4.3
Sorbitan mono-oleate (Span 80)	4.3
Sorbitan monostearate (Span 60)	4.7
Sorbitan monolaurate (Span 20)	8.6
Polysorbate 60 (polyoxyethylene sorbitan monostearate)	14.9
Polysorbate 80 (polyoxyethylene sorbitan mono-oleate) (Tween 80)	15.0
Polysorbate 20 (polyoxyethylene sorbitan mono-laurate) (Tween 20)	16.7
Potassium oleate	20.0
Sodium dodecyl (lauryl) sulphate	40.0

product. For an o/w emulsion, for example, the more polar the oil phase the more polar must be the emulgent system.

Table 23.3 gives the required emulgent HLB value for particular oil phases for both types of emulsion. If a formulation contains a mixture of oils, fats or waxes the total HLB required can be calculated. The following example of an o/w emulsion will show this.

Liquid paraffin	35%
Wool fat	1%
Cetyl alcohol	1%
Emulsifier system	5%
Water	to 100%

The total percentage of oil phase is 37 and the proportion of each is:

Liquid paraffin	$35/37 \times 100$	=	94.6%
Wool fat	$1/37 \times 100$	=	2.7%
Cetyl alcohol	$1/37 \times 100$	=	2.7%

The total required HLB number is obtained as follows:

Table 23.3 Required HLB values for a range of oils and waxes

	For a w/o emulsion	For an o/w emulsion
Beeswax	5	12
Cetyl alcohol	–	15
Liquid paraffin	4	12
Soft paraffin	4	12
Wool fat	8	10

Liquid paraffin (HLB 12)	$94.6/100 \times 12 = 11.4$
Wool fat (HLB 10)	$2.7/100 \times 10 = 0.3$
Cetyl alcohol (HLB 15)	$2.7/100 \times 15 = 0.4$
Total required HLB	**= 12.1**

From theoretical considerations, this particular formulation requires an emulgent blend of HLB 12.1 in order to produce the most stable emulsion. It must be realized, however, that the presence of other ingredients, particularly those that may partition into the oil phase, can also affect the required HLB value. It is therefore often necessary to prepare a series of emulsions using blends of a given pair of non-ionic emulsifying agents covering a wide range of HLB values. This is also important if the required HLB for an oil phase is not available. The HLB value of the emulgent blend giving the most stable emulsion is the required value for that oil phase.

Assuming that a blend of sorbitan mono-oleate (HLB 4.3) and polyoxyethylene sorbitan mono-oleate (HLB 15) is to be used as the emulsifying system, the proportions of each to be added to the emulsion to provide an HLB of 12.1 are calculated as follows.

Let A be the percentage concentration of the hydrophilic and B the percentage of the hydrophobic surfactants required to give a blend having an HLB value of x. Then:

$$A = \frac{100(x - \text{HLB of B})}{(\text{HLB of } A - \text{HLB of } B)} \quad \text{and} \quad B = 100 - A$$

In our example, therefore:

$$A = \frac{100(12.1 - 4.3)}{(15 - 4.3)} = 72.9$$

$B = 100 - 72.9 = 27.1$

Because the total percentage of emulgent blend in the formulation is 5, the percentage of each emulsifier will be:

Sorbitan mono-oleate	$5 \times 27.1/100 = 1.36$
Polyoxyethylene sorbitan mono-oleate	$5 - 1.36 = 3.64$

The series of trial emulsions can then be assessed for stability, based on the fact that the degree of creaming or separation is at a minimum at the optimal HLB value. Should several of the series show equally poor or equally good stability, resulting in an inability to choose a suitable HLB value, then the total emulgent concentration may be increased or reduced, respectively, and the manufacture of the series repeated.

Having determined the best HLB value for a given pair of emulgents, that value can now be used to

assess the suitability of other emulgent blends that may give a better emulsion than the one containing the emulgent used for the initial trials.

It must be remembered that, in choosing an emulsifier blend, the effect of chemical structure on the type of interfacial film must be taken into account. Condensed films are produced by emulgents having long, saturated hydrocarbon groups, thus providing maximum cohesion between adjacent molecules. In most cases it has been found that the most stable emulsions are formed when both emulsifying agents are of the same hydrocarbon chain length.

The use of phase inversion temperature

The use of the HLB system has several disadvantages, including the inability to take into account the effects of temperature, the presence of additives and the concentration of the emulsifier. It is possible to overcome some of these problems.

An o/w emulsion stabilized by non-ionic emulgents will, on heating, invert to form a w/o product. This is because, as the temperature increases, the HLB value of a non-ionic surfactant will decrease as it becomes more hydrophobic. At the temperature at which the emulgent has equal hydrophilic and hydrophobic tendencies (the phase inversion temperature) the emulsion will invert.

The stability of an emulsion has been related to the phase inversion temperature (PIT) of its emulsifying agent (see Chapter 6).

CLASSIFICATION OF EMULSIFYING AGENTS

The inclusion of an emulsifying agent or agents is necessary to facilitate actual emulsification during manufacture, and also to ensure emulsion stability during the shelf-life of the product.

The different methods by which emulsifying agents (also called emulsifiers or emulgents) exert their effects have been detailed in Chapter 6, but the one factor common to all of them is their ability to form an adsorbed film around the dispersed droplets between the two phases. There are many types of emulgent available, but for convenience they can be divided into two main classifications: synthetic or semisynthetic surface-active agents, and naturally occurring materials and their derivatives.

These divisions are quite arbitrary and some materials may justifiably be placed in more than one category.

Synthetic and semisynthetic surface-active agents

There are four main categories of these materials, depending on their ionization in aqueous solutions: anionic, cationic, non-ionic and amphoteric.

Anionic surfactants

In aqueous solutions these compounds dissociate to form negatively charged anions that are responsible for their emulsifying ability. They are widely used because of their cheapness, but because of their toxicity are only used for externally applied preparations.

Alkali metal and ammonium soaps Emulgents in this group consist mainly of the sodium, potassium or ammonium salts of long-chain fatty acids, such as:

sodium stearate $C_{17}H_{35}COO^- Na^+$

They produce stable o/w emulsions but may in some instances require the presence of an auxiliary non-ionic emulsifying agent in order to form a complex monomolecular film at the oil/water interface. Because in acidic conditions, these materials will precipitate out as the free fatty acids, they are most efficient in an alkaline medium.

This type of emulgent can also be formed in situ during the manufacture of the product by reacting an alkali such as potassium, sodium or ammonium hydroxide with a fatty acid. The latter may be a constituent of a vegetable oil. Oleic acid and ammonia, for example, are reacted together to form the soap responsible for stabilizing White Liniment.

These emulgents are incompatible with polyvalent cations, often causing phase reversal, and it is therefore essential that deionized water is used in their preparation.

Soaps of divalent and trivalent metals Although many different divalent and trivalent salts of fatty acids exist, and would produce satisfactory emulsions, only the calcium salts are commonly used. They are often formed in situ during preparation of the product by interacting the appropriate fatty acid with calcium hydroxide. For example, oleic acid is reacted with calcium hydroxide to produce calcium oleate, which is the emulsifying agent for both Zinc Cream BP and some formulations of oily calamine lotion.

These emulgents will only produce w/o emulsions.

Amine soaps A number of amines form salts with fatty acids. One of the most important of those used is based on triethanolamine $N(CH_2CH_2OH)_3$ and is widely used in both pharmaceutical and cosmetic products. For example, triethanolamine stearate

forms stable o/w emulsions and is usually made in situ by a reaction between triethanolamine and the appropriate fatty acid. Although these emulgents are usually pH neutral they are still restricted to externally used preparations. They are also incompatible with acids and high concentrations of electrolytes.

Sulphated and sulphonated compounds The alkyl sulphates have the general formula $ROSO_3^-M^+$, where R represents a hydrocarbon chain and M^+ is usually sodium or triethanolamine. An example is sodium lauryl sulphate, which is widely used to produce o/w emulsions. Because of its high water solubility and its inability to form condensed films at the oil/water interface, it is always used in conjunction with a non-ionic oil-soluble emulsifying agent in order to produce a complex condensed film. It is used with cetostearyl alcohol to produce Emulsifying Wax, which stabilizes such preparations as Aqueous Cream and Benzyl Benzoate Application.

Sulphonated compounds are much less widely used as emulgents. Materials of this class include sodium dioctylsulphosuccinate, and are more often used as wetting agents or for their detergency.

Cationic surfactants

In aqueous solutions these materials dissociate to form positively charged cations that provide the emulsifying properties. The most important group of cationic emulgents consists of the quaternary ammonium compounds. Although these materials are widely used for their disinfectant and preservative properties, they are also useful o/w emulsifiers. Like many anionic emulgents, if used on their own they will produce only poor emulsions, but if used with non-ionic oil-soluble auxiliary emulgents they will form stable preparations.

Because of the toxicity of cationic surfactants they tend to be used only for the formulation of antiseptic creams, where the cationic nature of the emulgent is also responsible for the product's antiseptic properties.

Cationic emulsifying agents are incompatible with anionic surface-active agents and polyvalent anions, and are unstable at high pH.

Cetrimide The most useful of these cationic emulgents is cetrimide (cetyl trimethylammonium bromide) $CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$. Cetrimide is used at a concentration of 0.5% with 5% cetostearyl alcohol for the formulation of Cetrimide Cream BP.

Non-ionic surfactants

These products range from oil-soluble compounds stabilizing w/o emulsions to water-soluble materials giving o/w products. It is usual for a combination of a water-soluble with an oil-soluble emulgent to be used in order to obtain the complex interfacial film necessary for optimum emulsion stability. Non-ionic emulgents are particularly useful because of their low toxicity and irritancy; some can therefore be used for orally and parenterally administered preparations. They also have a greater degree of compatibility with other materials than do anionic or cationic emulgents, and are less sensitive to changes in pH or to the addition of electrolytes. They do, however, tend to be more expensive.

Being non-ionic, the dispersed globules may not possess a significant charge density. To reduce the tendency for coalescence to occur in an oil-in-water emulsion, it is necessary that the polar groups be well hydrated and/or sufficiently large to prevent close approach of the dispersed droplets in order to compensate for the lack of charge.

Most non-ionic surfactants are based on:

- a fatty acid or alcohol (usually with 12–18 carbon atoms), the hydrocarbon chain of which provides the hydrophobic moiety;
- an alcohol (-OH) and/or ethylene oxide grouping ($-OCH_2CH_2-$), which provide the hydrophilic part of the molecule.

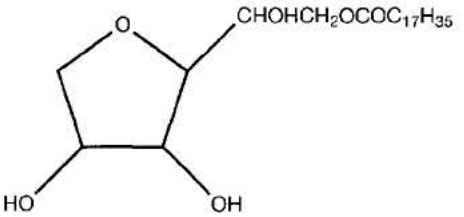
By varying the relative proportions of the hydrophilic and hydrophobic groupings many different products can be obtained.

If the hydrophobic part of the molecule predominates, then the surfactant will be oil-soluble. It will not concentrate at the oil/water interface but rather tend to migrate into the oil phase. Similarly, a water-soluble surfactant will migrate into the aqueous phase and away from the oil/water interface. The best type of non-ionic surfactant to use is one with an equal balance of hydrophobic and hydrophilic groupings. An alternative would be to use two emulgents, one hydrophilic and one hydrophobic. The cohesion between their hydrocarbon chains will then hold both types at the oil/water interface.

Glycol and glycerol esters Glyceryl monostearate (a polyhydric alcohol fatty acid ester) is a strongly hydrophobic material that produces weak w/o emulsions. The addition of small amounts of sodium, potassium or triethanolamine salts of suitable fatty acids will produce a 'self-emulsifying' glyceryl monostearate, which is a useful o/w emulsifier. Self-emulsifying monostearin is glyceryl monostearate to which anionic soaps (usually oleate or stearate) have been added. This combination is used to stabilize Hydrocortisone Lotion.

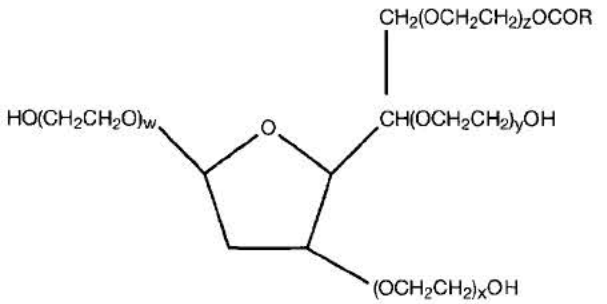
Other polyhydric alcohol fatty acid esters are also available either in the pure form or in the 'self-emulsifying' form containing small proportions of a primary emulsifier, and include glyceryl mono-oleate, diethylene glycol monostearate and propylene glycol mono-oleate.

Sorbitan esters These are produced by the esterification of one or more of the hydroxyl groups of sorbitan with either lauric, oleic, palmitic or stearic acids. The structure of sorbitan monostearate is shown below.



This range of surfactants exhibits lipophilic properties and tends to form w/o emulsions. They are, however, much more widely used with polysorbates to produce either o/w or w/o emulsions.

Polysorbates Polyethylene glycol derivatives of the sorbitan esters give us polysorbates. These have the general formula:



where R represents a fatty acid chain. Variations in the type of fatty acid used and in the number of oxyethylene groups in the polyethylene glycol chains produce a range of products of differing oil and water solubilities. Polyoxyethylene 20 sorbitan mono-oleate, for example, contains 20 oxyethylene groups in the molecule. This number must not be confused with the one given as part of the official name (Polysorbate 80) or in the trade name (Tween 80), which is included in order to identify the type of fatty acid in the molecule.

Polysorbates are generally used in conjunction with the corresponding sorbitan ester to form a complex condensed film at the oil/water interface (see Formulation by the HLB method, earlier).

Other non-ionic oil-soluble materials, such as glyceryl monostearate, cetyl or stearyl alcohol or propylene glycol monostearate, can be incorporated with polysorbates to produce 'self-emulsifying' preparations. For example, Polawax contains cetyl alcohol with a polyoxyethylene sorbitan ester.

Polysorbates are compatible with most anionic, cationic and non-ionic materials. They are pH neutral and are stable to the effects of heat, pH change and high concentrations of electrolyte. Their low toxicity renders them suitable for oral use and some are also used in parenteral preparations. They have the disadvantage, however, of an unpleasant taste, and care must be taken when selecting a suitable preservative as many are inactivated by complexation with polysorbates.

Fatty alcohol polyglycol ethers These are condensation products of polyethylene glycol and fatty alcohols, usually cetyl or cetostearyl:

$$ROH + (CH_2CH_2O)_n \rightarrow RO(CH_2CH_2O)_nH$$

where R is a fatty alcohol chain.

Perhaps the most widely used is macrogol cetostearyl ether (22) or cetomacrogol 1000, which is polyethylene glycol monocetyl ether. This is a very useful water-soluble o/w emulgent, but because of its high water solubility it is necessary to include an oil-soluble auxiliary emulsifier when formulating emulsions. Cetomacrogol Emulsifying Ointment includes cetomacrogol 1000 and cetostearyl alcohol and is used to stabilize cetomacrogol creams.

They can also be produced with shorter polyoxyethylene groups as lipophilic w/o emulsifiers. Combinations of lipophilic and hydrophilic ethers can be used together to produce stable emulsions.

These materials can be salted out by the addition of high concentrations of electrolyte, but are stable over a wide pH range.

Fatty acid polyglycol esters The stearate esters or polyoxyl stearates are the most widely used of this type of emulgent. Polyoxyethylene 40 stearate (in which 40 represents the number of oxyethylene units) is a water-soluble material often used with stearyl alcohol to give o/w emulsions.

Poloxalkols Poloxalkols are polyoxyethylene/polyoxypropylene copolymers with the general formula:

$$OH(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_a$$

and comprise a very large group of compounds, some of which are used as emulsifying agents for intravenous fat emulsions.

Higher fatty alcohols The hexadecyl (cetyl) and octadecyl (stearyl) members of this series of saturated aliphatic monohydric alcohols are useful auxiliary emulsifying agents. Part of their stabilizing effect

comes from their ability to increase the viscosity of the preparation, thereby retarding creaming. Cetostearyl alcohol will also form complex interfacial films with hydrophilic surface-active agents such as sodium lauryl sulphate, cetrimide or cetomacrogol 1000, and so stabilize o/w emulsions.

Amphoteric surfactants

This type possesses both positively and negatively charged groups, depending on the pH of the system. They are cationic at low pH and anionic at high pH. Although they are not widely used as emulsifying agents, one example, lecithin, is used to stabilize intravenous fat emulsions.

Naturally occurring materials and their derivatives

Naturally occurring materials often suffer from two main disadvantages: they show considerable batch-to-batch variation in composition and hence in emulsifying properties, and many are susceptible to bacterial or mould growth. For these reasons they are not widely used in manufactured products requiring a long shelf-life, but rather for extemporaneously prepared emulsions designed for use within a few days of manufacture.

Polysaccharides

The most important emulsifying agent in this group is acacia. This stabilizes o/w emulsions by forming a strong multimolecular film round each oil globule, and so coalescence is retarded by the presence of a hydrophilic barrier between the oil and water phases.

Because of its low viscosity, creaming will occur readily, and therefore a suspending agent such as tragacanth or sodium alginate can also be included. Because of its sticky nature the use of acacia is limited to products for internal use.

Semisynthetic polysaccharides

In order to reduce the problems associated with batch-to-batch variation, semisynthetic derivatives are available as o/w emulgents or stabilizers.

Several grades of methylcellulose and carmellose sodium are available and exert their action in a similar way to that of acacia.

Methylcellulose 20, for example, is used at a concentration of 2% to stabilize Liquid Paraffin Oral Emulsion.

Sterol-containing substances

Beeswax, wool fat and wool alcohols are all used in the formulation of emulsions. Beeswax is used mainly in cosmetic creams of both o/w and w/o type, in conjunction with borax. Because of the systemic toxicity of boric acid and its salts, however, the use of beeswax/borax preparations is limited, although beeswax is used as a stabilizer for w/o creams.

Wool fat (anhydrous lanolin) consists chiefly of normal fatty alcohols with fatty acid esters of cholesterol and other sterols. It will form w/o emulsions of low dispersed phase concentration, and it can also be incorporated for its emollient properties. Some individuals exhibit sensitization to this material and, because of its characteristic odour and the need to incorporate antioxidants, it is not widely used. It is, however, to be found in low concentrations in many ointments, where its water-absorbing properties are of great value. It can be employed as an emulsion stabilizer with a primary emulsifying agent, for example with calcium oleate in oily calamine lotion, with beeswax in Proflavine Cream, and with cetostearyl alcohol in Zinc Cream and ichthammol cream.

Because wool fat has some ideal properties, attempts have been made to improve its other, less desirable, characteristics by physical and chemical modification. Processes including hydrogenation and fractionation have been carried out with some success. It has also been converted, by a reaction with ethylene oxide, to give a range of polyoxyethylene lanolin derivatives. These non-ionic products are mainly water soluble and are used as o/w emulgents possessing the properties of emollience.

The principal emulsifying agent in wool fat is wool alcohols, which consists mainly of cholesterol together with other alcohols. It is an effective w/o emulgent, being more powerful than wool fat, and is used in the formulation of Hydrous Ointment. It is also incorporated as Wool Alcohols Ointment into other ointment bases which, although not emulsions, will readily mix with aqueous skin secretions and easily wash off the skin. Wool alcohols does not have the same strong odour as wool fat but does require the presence of an antioxidant.

Finely divided solids

Certain finely divided solids can be adsorbed at the oil/water interface, forming a coherent film that physically prevents coalescence of the dispersed globules. If the particles are preferentially wetted by the aqueous phase then o/w products will result, whereas preferential wetting by the oil will produce w/o emulsions.

Montmorillonite clays (such as bentonite and aluminium magnesium silicate) and colloidal silicon dioxide are used mainly for external use. Aluminium and magnesium hydroxides are also used internally. For example, Liquid Paraffin and Magnesium Hydroxide Oral Emulsion BP is stabilized by the incorporation of the magnesium hydroxide.

Other formulation additives

Buffers

The inclusion of buffers (see Chapters 3, 21 and 35) may be necessary to maintain chemical stability, control tonicity or ensure physiological compatibility. It must be remembered, however, that the addition of electrolytes may have profound effects on the physical stability of suspensions and emulsions.

Density modifiers

From a qualitative examination of Stokes' law (Chapter 6) it can be seen that if the disperse and continuous phases both have the same densities then sedimentation or creaming will not occur. Minor modifications to the aqueous phase of a suspension or emulsion by incorporating sucrose, dextrose, glycerol or propylene glycol can be achieved, but because of the differing coefficients of expansion this can only be possible over a small temperature range.

Humectants

Glycerol, polyethylene glycol and propylene glycol are examples of suitable humectants that can be incorporated at concentrations of about 5% into aqueous suspensions for external application. They are used to prevent the product from drying out after application to the skin.

They can also be added to an emulsion formulation in order to reduce the evaporation of the water, either from the packaged product when the closure is removed or from the surface of the skin after application. High concentrations, if used topically, may actually remove moisture from the skin, thereby dehydrating it.

Antioxidants

Before including an antioxidant in emulsion formulations, it is essential to ensure that its use is not restricted in whichever country it is desired to sell the product. In Britain, butylated hydroxyanisole (BHA) is widely used for the protection of fixed oils and fats at concentrations of up to 0.02% and for some essential oils up to 0.1%. A similar antioxidant is butylated hydroxytoluene (BHT), which is recommended as an alternative to tocopherol at a concentration of 10 ppm to stabilize liquid paraffin. Other antioxidants widely used for emulsion formulation include the propyl, octyl and dodecyl esters of gallic acid, recommended for use at concentrations up to 0.001% for fixed oils and fats and up to 0.1% for essential oils.

The efficiency of an antioxidant in a product will depend on many factors, including its compatibility with other ingredients, its oil/water partition coefficient, the extent of its solubilization within micelles of the emulgent, and its sorption on to the container and its closure. It must be realized, therefore, that the choice of antioxidant and the concentration at which it is to be used can only be determined by testing its effectiveness in the final product and in the package in which the product is to be sold.

Flavours, colours and perfumes

The use of these ingredients is discussed in Chapter 21 and the information there will be directly applicable to suspension and emulsion formulation.

Adsorption of these materials on to the surfaces of the dispersed phase of a suspension may occur, and because of the high surface area of the dispersed powders in this type of formulation, their effective concentrations in solution may be significantly reduced. The finer the degree of subdivision of the disperse phase, the paler may appear the colour of the product for a given concentration of dye.

It must also be realized that the inclusion of these adjuvants may alter the physical characteristics of both suspensions and emulsions. Either the presence of electrolytes or their effect on pH can influence the degree of flocculation.

Sweetening agents

Suitable sweeteners are discussed in Chapter 21. High concentrations of sucrose, sorbitol or glycerol, which will exhibit Newtonian properties, may adversely affect the rheological properties of the suspension. Synthetic sweeteners may be salts and can affect the degree of flocculation.

Preservation of suspensions and emulsions

Preservation of suspensions

The section covering the preservation of emulsions is applicable also to suspension formulation. It is

essential that a suitable preservative be included, particularly if naturally occurring materials are to be used. This is to prevent the growth of micro-organisms that may be present in the raw material and/or introduced into the product during use. Some of the natural products, particularly if they are to be applied to broken skin, should be sterilized before use. Bentonite, for example, may contain *Clostridium tetani* but can be sterilized by heating the dry powder at 160°C for 1 hour or by autoclaving aqueous dispersions.

As with emulsion formulation, care must be taken to ascertain the extent of inactivation, if any, of the preservative system caused by interaction with other excipients. Solubilization by wetting agents, interaction with polymers or adsorption on to suspended solids, particularly kaolin or magnesium trisilicate, may reduce the availability of preservatives.

Preservation of emulsions

Problems associated with the growth of micro-organisms in pharmaceutical products are discussed in Chapter 43. Those microbiological factors of specific importance to the stability of emulsions are discussed later in this chapter. The necessity of including a preservative in an emulsion formulation is discussed below.

Unfortunately there is no theoretical way of choosing a suitable preservative system, the only reliable methods being based on the results of suitable challenge tests. These methods of testing preservative activity are given in official compendia, but essentially involve the addition to the test products of a mixture of Gram-positive and Gram-negative bacteria, yeasts and moulds, and comparing their survival with a control sample containing no preservative.

The desirable features of a preservative suitable for use in an emulsion include:

- a wide spectrum of activity against all bacteria, yeasts and moulds;
- bactericidal rather than bacteristatic activity. A preservative having a minimal bacteristatic activity may lose it if any physical or chemical changes occur in the system;
- freedom from toxic, irritant or sensitizing activity;
- high water solubility. Because the growth of microorganisms occurs in the aqueous phase, it is important that the preservative has a low oil/water partition coefficient. The more polar the oil phase, the more difficult it is to preserve the product adequately, owing to the solubility of the preservative in both phases. If the preservative is more soluble in oil than in water, then increasing the proportion of oil will decrease the aqueous phase concentration. Allowance must be made for this when choosing the phase–volume ratios;
- compatibility with the other ingredients and with the container. Certain preservatives are incompatible with particular groups of emulsifying agent. Phenols and the esters of *p*-hydroxybenzoic acid, for example, will complex with some non-ionic emulgents, owing to either a reaction with oxyethylene groups or solubilization within micelles of excess surfactant. In many cases it is possible, by chemical assay, to detect the correct concentration of preservative in the product even though some of it may not be available for antimicrobial activity. If some of the added preservative has been inactivated it may be possible to overcome this problem by increasing the amount of preservative in the product to give a satisfactory concentration of free preservative in the aqueous phase. It is important to ensure that, during manufacture, the preservative is added after the emulgent has concentrated at the oil/water interface;
- stability and effectiveness over a wide range of pH and temperatures;
- freedom from colour and odour;
- retention of activity in the presence of large numbers of microorganisms. Uptake of preservative by bacterial cells may deplete the concentration of preservative in solution, thereby rendering it insufficient to maintain adequate bactericidal activity.

Because of the complex systems involved and the many factors to be taken into consideration, it is necessary to test the efficiency of a new preservative in the finished product and container by suitable challenge testing procedures.

The most widely used preservatives in emulsions include benzoic and sorbic acid and their salts, *p*-hydroxybenzoic acid esters, chlorocresol, phenoxyethanol, bronopol, quaternary ammonium compounds and, to a lesser extent, organic mercurials. Because of the irritancy and toxicity of certain preservatives, the initial choice will depend on the route of administration of the product. Further details of the use of preservatives in emulsions can be found in Chapter 42.

It must be realized that no single preservative exhibits all of the desirable properties outlined earlier. In many cases a combination is required, the most widely used being a mixture of methyl and propyl *p*-hydroxybenzoates at a ratio usually of 10:1.

PHYSICAL STABILITY OF SUSPENSIONS

The physical stability of a suspension is normally assessed by the measurement of its rate of sedimentation, the final volume or height of the sediment, and the ease of redispersion of the product.

The first two parameters can be assessed easily by a measurement of the total initial volume or height of the suspension (V_0) and the volume or height of the sediment (V_t), as shown in Figure 23.1. By plotting the value of V_t/V_0 against time for a series of trial formulations (all initial values will equal unity), it can be seen, by an assessment of the slope of each line, which suspension shows the slowest rate of sedimentation. When the value of V_t/V_0 becomes constant this indicates that sedimentation has ceased.

Alternatively, the term flocculation value can be used, which is a ratio of the final volume or height of the sediment and the volume or height of the fully sedimented cake of the same system which has been deflocculated.

Attempts have also been made to equate the zeta potential of the suspended particles with the physical stability – particularly the degree of flocculation – of the system using electrophoresis.

The ease of redispersion of the product can be assessed qualitatively by simply agitating the product in its container. The use of a mechanical shaker will eliminate variations in shaking ability.

PHYSICAL STABILITY OF EMULSIONS

A stable emulsion is one in which the dispersed globules retain their initial character and remain uniformly distributed throughout the continuous phase. Various types of deviation from this ideal behaviour can occur. Explanations for emulsion stability have been given in Chapter 6. This section will concentrate on methods of improving emulsion stability in practice.

Creaming and its avoidance

This is the separation of an emulsion into two regions, one of which is richer in the disperse phase than the other. A simple example is the creaming of milk, when fat globules slowly rise to the top of the product. This is not a serious instability problem as a uniform dispersion can be reobtained simply by shaking the emulsion. It is, however, undesirable because of the increased likelihood of coalescence of the droplets, owing to their close proximity to each other. A creamed emulsion is also inelegant and, if the emulsion is not shaken adequately, there is a risk of the patient obtaining an incorrect dosage.

Consideration of the qualitative application of Stokes' law will show that the rate of creaming can be reduced by the following methods.

Production of an emulsion of small droplet size

This factor usually depends on the method of manufacture. An efficient emulsifying agent will not only stabilize the emulsion but also facilitate the actual emulsification process to give a product of fine globule size.

Increase in the viscosity of the continuous phase

Many auxiliary emulsifying agents, in particular the hydrophilic colloids, are viscosity enhancers and this property is part of their emulsifying capability. For example, the inclusion of methylcellulose will reduce the mobility of the dispersed droplets in an o/w emulsion. The addition of soft paraffin will have the same effect on water droplets in a w/o emulsion.

Storage of the product at a low temperature (but above freezing point) will increase the viscosity of the continuous phase and also reduce the kinetic energy of the system. This will decrease the rate of migration of the globules of the disperse phase. It is unwise, however, to rely solely on this method of controlling creaming, as storage conditions after the product is sold are outside the control of the manufacturer.

Reduction in the density difference between the two phases

Creaming could be prevented altogether if the densities of the two phases were identical. In practice this method is never used, as it could only be achieved over a very narrow temperature range owing to differences in the coefficients of expansion between different ingredients.

Control of disperse phase concentration

It is not easy to stabilize an emulsion containing less than 20% disperse phase, as creaming will readily occur. A higher disperse phase concentration would result in a hindrance of movement of the droplets and hence in a reduction in rate of creaming.

Although it is theoretically possible to include as much as 74% of an internal phase, it is usually found that at about 60% concentration phase inversion occurs.

Finally, it must be realized that some of the factors above are interrelated. For example, homogenization of the emulsion would decrease globule size and, by thus increasing their number, increase the viscosity of the product.

Flocculation prevention

Flocculation involves the aggregation of the dispersed globules into loose clusters within the emulsion. The individual droplets retain their identities but each cluster behaves physically as a single unit. This, as we have already seen, would increase the rate of creaming. As flocculation must precede coalescence, any factor preventing or retarding flocculation would therefore maintain the stability of the emulsion.

Flocculation in the secondary minimum (see Fig. 6.3) occurs readily and cannot be avoided. Redispersion can easily be achieved by shaking. Primary minimum flocculation, however, is more serious and redispersion is not so easy.

The presence of a high charge density on the dispersed droplets will ensure the presence of a high energy barrier, and thus reduce the incidence of flocculation in the primary minimum. Care must be taken to ensure that the effects of any ions in the product are taken into consideration very early in the formulation process. This is particularly important when formulating emulsions for parenteral nutrition which contain high levels of electrolytes (Washington 1990).

Coalescence (breaking, cracking)

The coalescence of oil globules in an o/w emulsion is resisted by the presence of a mechanically strong adsorbed layer of emulsifier around each globule. This is achieved by the presence of either a condensed mixed monolayer of lipophilic and hydrophilic emulgents, or a multimolecular film of a hydrophilic material. Hydration of either of these types of film will hinder the drainage of water from between adjacent globules which is necessary prior to coalescence. As two globules, approach each other their close proximity causes their adjacent surfaces to flatten. As a change from a sphere to any other shape results in an increase in surface area and hence in total surface free energy, this globule distortion will be resisted and drainage of the film of continuous phase from between the two globules will be delayed.

The presence of long, cohesive hydrocarbon chains projecting into the oil phase will prevent coalescence in a w/o emulsion.

CHEMICAL INSTABILITY OF EMULSIONS

Although it is not possible to list every incompatibility, the following general points will illustrate the more common chemical problems that can cause the coalescence of an emulsion.

It is necessary to ensure that any emulgent system used is not only physically but also chemically compatible with the active agent and with the other emulsion ingredients. Ionic emulsifying agents, for example, are usually incompatible with materials of opposite charge. Anionic and cationic emulgents are thus mutually incompatible.

It has already been demonstrated that the presence of electrolyte can influence the stability of an emulsion either by:

- reducing the energy of interaction between adjacent globules, or
- a salting-out effect, by which high concentrations of electrolytes can strip emulsifying agents of their hydrated layers and so cause their precipitation.

In some cases phase inversion may occur rather than demulsification. If, for example, a sodium soap is used to stabilize an o/w emulsion, then the addition of a divalent electrolyte such as calcium chloride may form the calcium soap, which will stabilize a w/o emulsion.

Emulgents may also be precipitated by the addition of materials in which they are insoluble. It may be possible to precipitate hydrophilic colloids by the addition of alcohol. For this reason care must therefore be taken if tinctures are to be included in emulsion formulations.

Changes in pH may also lead to the breaking of emulsions. Sodium soaps may react with acids to produce the free fatty acid and the sodium salt of the acid. Soap-stabilized emulsions are therefore usually formulated at an alkaline pH.

Oxidation

Many of the oils and fats used in emulsion formulation are of animal or vegetable origin and can be susceptible to oxidation by atmospheric oxygen or by the action of microorganisms. The resulting rancidity is manifested by the formation of

degradation products of unpleasant odour and taste. These problems can also occur with certain emulsifying agents, such as wool fat or wool alcohols. Oxidation of microbiological origin is controlled by the use of antimicrobial preservatives, and atmospheric oxidation by the use of reducing agents or, more usually, antioxidants. Some examples are mentioned earlier in this chapter.

Microbiological contamination

The contamination of emulsions by microorganisms can adversely affect the physicochemical properties of the product, causing such problems as gas production, colour and odour changes, hydrolysis of fats and oils, pH changes in the aqueous phase, and breaking of the emulsion. Even without visible signs of contamination an emulsion can contain many bacteria and, if these include pathogens, may constitute a serious health hazard. Most fungi and many bacteria will multiply readily in the aqueous phase of an emulsion at room temperature, and many moulds will also tolerate a wide pH range. Some of the hydrophilic colloids, which are widely used as emulsifying agents, may provide a suitable nutritive medium for use by bacteria and moulds. Species of the genus *Pseudomonas* can utilize polysorbates, aliphatic hydrocarbons and compounds. Some fixed oils, including arachis oil, can be used by some *Aspergillus* and *Rhizopus* species, and liquid paraffin by some species of *Penicillium*.

A few emulgents, particularly those from natural sources, may introduce heavy contamination into products in which they are used. Because bacteria can reproduce in resin beds, deionized water may be unsatisfactory and even distilled water, if incorrectly stored after collection, can be another source of contamination. Oil-in-water emulsions tend to be more susceptible to microbial spoilage than water-in-oil products as, in the latter case the continuous oil phase acts as a barrier to the spread of microorganisms throughout the product, and the less water there is present the less growth there is likely to be.

It is therefore, necessary to include an antimicrobial agent to prevent the growth of any microorganisms that might contaminate the product. Suitable candidates are discussed in Chapter 42.

Adverse storage conditions

Adverse storage conditions may also cause emulsion instability. It has already been explained that an increase in temperature will cause an increase in the rate of creaming, owing to a fall in apparent viscosity of the continuous phase. The temperature increase will also cause an increased kinetic motion, both of the dispersed droplets and of the emulsifying agent at the oil/water interface. This effect on the disperse phase will enable the energy barrier to be easily surmounted and thus the number of collisions between globules will increase. Increased motion of the emulgent will result in a more expanded monolayer, and so coalescence is more likely. Certain macromolecular emulsifying agents may also be coagulated by an increase in temperature.

At the other extreme, freezing of the aqueous phase will produce ice crystals that may exert unusual pressures on the dispersed globules and their adsorbed layer of emulgent. In addition, dissolved electrolyte may concentrate in the unfrozen water, thus affecting the charge density on the globules. Certain emulgents may also precipitate at low temperatures.

The growth of microorganisms within the emulsion can cause deterioration and it is therefore essential that these products are protected as far as possible from the ingress of microorganisms during manufacture, storage and use, and that they contain adequate preservatives.

STABILITY TESTING OF EMULSIONS

Methods of assessing stability

Macroscopic examination

The physical stability of an emulsion can be assessed by an examination of the degree of creaming or coalescence occurring over a period of time. This is carried out by calculating the ratio of the volume of the creamed or separated part of the emulsion and the total volume. These values can be compared for different products.

Globule size analysis

If the mean globule size increases with time (coupled with a decrease in globule numbers), it can be assumed that coalescence is the cause. It is therefore possible to compare the rates of coalescence for a variety of emulsion formulations by this method. Microscopic examination or electronic particle counting devices, such as the Coulter counter, or laser diffraction sizing are most widely used.

Viscosity changes

It has already been shown that many factors influence the viscosity of emulsions. Any variation in globule size or number, or in the orientation or migration of emulsifier over a period of time, may be detected by a change in apparent viscosity. Suitable methods and equipment are detailed in Chapter 4.

In order to compare the relative stabilities of a range of similar products it is often necessary to speed up the processes of creaming and coalescence. This can be achieved in one of the following ways.

Accelerated stability tests

To assess the physical stability of suspensions and emulsions macroscopic examination and measurement of apparent viscosity are of value. In addition, for emulsions, microscopic evaluation of globule size distribution and numbers will provide further evidence of changes in physical stability.

Storage at adverse temperatures

An assessment of these parameters at elevated temperatures for emulsions and coarse suspensions would give a speedier indication of a rank order of degree of instability, but it is essential to correlate these results with those taken from suspensions stored at ambient temperatures.

Temperature cycling By exaggerating the temperature fluctuations to which any product is subjected under normal storage conditions, it may be possible to compare the physical stabilities of a series of suspensions or emulsions. Temperature cycles consisting of storage for several hours at about 40°C, followed by refrigeration or freezing until instability becomes evident, have been used successfully. The continual formation and melting of small ice crystals will disrupt the adsorbed layer of emulgent at the oil/water interface, and any weakness in the structure of the film will quickly become apparent. Similarly, normal temperature fluctuations can be used, but at increased frequencies of only a few minutes at each extreme. This method of accelerated stability testing is particularly useful for the assessment of crystal growth in suspensions. Measurement of particle size is usually carried out microscopically, by laser diffraction or by use of a Coulter counter. It is of course important to ensure that the suspension is deflocculated to ensure that each individual particle is measured, rather than each floccule.

Centrifugation

A qualitative examination of Stokes' law (Chapters 4 and 10) would indicate centrifugation to be a suitable method for artificially increasing the rate of sedimentation of a suspension. Again, it is not always possible to predict accurately the behaviour of such a system when stored under normal conditions from data obtained after this type of accelerated testing. The process of centrifugation may destroy the structure of a flocculated system that would remain intact under normal storage conditions. The sediment formed would become tightly packed and difficult to redisperse, whether or not the initial suspension was flocculated or deflocculated. This method may, however, give a useful indication of the relative stabilities of a series of trial products, particularly if used at speeds no faster than 200–300 rpm.

Rheological assessment

Although apparent viscosity measurements are also used as a tool to assess physical stability, the high shear rates involved may also destroy the structure of a suspension or emulsion. Very low rates of shear, using for example the Brookfield viscometer with Helipath stand, can give an indication of the change in the structure of the system after various storage times. For suspensions it may be possible to combine the results from sedimentation techniques with those from rheological assessments.

A measurement of the residual apparent viscosity, after breaking down the structure of the suspension, can be used as a routine quality control procedure after manufacture.

MANUFACTURE OF SUSPENSIONS

It is important to ensure initially that the powder to be suspended is in a suitably fine degree of subdivision in order to ensure adequate bioavailability, minimum sedimentation rate and impalpability. Suitable size reduction equipment and the relative merits of wet and dry milling are detailed in Chapter 11.

For the extemporaneous preparation of suspensions on a small scale, the powdered drug can be mixed with the suspending agent and some of the vehicle using a pestle and mortar. It may also be necessary, at this stage, to include a wetting agent to aid dispersion. Other soluble ingredients should then be dissolved in another portion of the vehicle,

mixed with the concentrated suspension and then made up to volume.

It is often preferable, particularly on a larger scale, to make a concentrated dispersion of the suspending agent first. This is best accomplished by adding the material slowly to the vehicle while mixing. Suitable mixers are described under Manufacture of emulsions, but can include either an impeller type of blender or a turbine mixer. This stage is important, as it is necessary to ensure that agglomerates of the suspending agent are fully broken up. If they are not, then the surface of each agglomerate may gel and cause the powder inside to remain non-wetted. Very intense shearing, however, can destroy the polymeric structure of the suspending agent, and it may be better to use milder shearing and then allow the dispersion to stand until full hydration has been achieved. This may be instantaneous or may, as with tragacanth, take several hours. If the suspending agent is blended with one of the water-soluble ingredients, such as sucrose, this will also aid dispersion.

The drug to be suspended is then added in the same way, along with the wetting agent. For very hydrophobic drugs, wetting may be facilitated by mixing under reduced pressure. This has the additional advantage of de-aerating the product and thus improving its appearance. Other ingredients should now be added, preferably dissolved in a portion of the vehicle, and the whole made up to volume if necessary. Finally, homogenization (see under Manufacture of emulsions) would ensure complete dispersion of the drug and the production of a smooth and elegant preparation.

It is also possible, though much less widely used, to suspend an insoluble drug by precipitating it from a solution. This can be accomplished either by double decomposition or, if it is a weak acid or a weak base, by altering the pH of its solution or by precipitating the drug from a water-miscible solvent on the addition of water. This method may be of use if the drug is required to be sterile but is degraded by heat or irradiation. A soluble form of the drug is dissolved in a suitable vehicle, sterilized by filtration and then precipitated to form a suspension.

In normal circumstances aqueous suspensions can be autoclaved, as long as the process does not adversely affect either physical or chemical stability.

MANUFACTURE OF EMULSIONS

It has already been explained that the smaller the globules of the disperse phase, the slower will be the rate of creaming in an emulsion. The size of these globules can also affect the viscosity of the product, and in general it has been found that the best emulsions with respect to physical stability and texture exhibit a mean globule diameter of between 0.5 and 2.5 μm. The choice of suitable equipment for the emulsification process depends mainly on the intensity of shearing required to produce this optimum particle size. Other considerations, however, include the volume and viscosity of the emulsion and the interfacial tension between the oil and the water. The presence of surfactants, which will reduce interfacial tension, will aid the process of emulsification as well as promoting emulsion stability.

In many cases simple blending of the oil and water phases with a suitable emulgent system may be sufficient to produce satisfactory emulsions. Further processing using a homogenizer can also be carried out to reduce globule size still further. The initial blending may be accomplished on a small scale by the use of a pestle and mortar or by using a mixer fitted with an impeller type of agitator, the size and type of which will depend primarily on the volume and viscosity of the emulsified product.

A more intense rate of shearing can be achieved using a turbine mixer such as the Silverson mixer–homogenizer. In this type of machine the short, vertical or angled rotor blades are enclosed within a stationary perforated ring and connected by a central rod to a motor. The liquids are therefore subjected to intense shearing, caused initially by the rotating blades, and then by the forced discharge through the perforated ring. Different models are available for a variety of batch sizes up to several thousand litres, and can include inline models.

The mixing vessel may also be fitted with baffles in order to modify the circulation of the liquid, and may be jacketed so that heating or cooling can be applied.

Homogenizers are often used after initial mixing to enable smaller globule sizes to be produced. They all work on the principle of forced discharge of the emulsion under pressure through fine interstices, formed by closely packed metal surfaces, in order to provide an intense shearing action.

If two immiscible liquids are subjected to ultrasonic vibrations, alternate regions of compression and rarefaction are produced. Cavities are then formed in the regions of rarefaction, which then collapse with considerable force causing emulsification. The required frequency of vibration is usually produced electrically, but mechanical methods are also available. Unfortunately this method of emulsification is limited to small-scale production.

Colloid mills are also suitable for the preparation of emulsions on a continuous basis. The intense shearing of the product between the rotor and the stator, which can be variably separated, will produce emulsions of very small globule size.

It is important to ensure that methods of manufacture developed on a laboratory scale can be easily extended to large-scale production, and without any change in the quality of the product.

During manufacture it is usual to add the disperse phase to the continuous phase during the initial mixing. The other ingredients are dissolved, prior to mixing, in the phase in which they are soluble. This is particularly important when making w/o emulsions. Oil-in-water emulsions, however, are sometimes made by the phase-inversion technique, in which the aqueous phase is slowly added to the oil phase during mixing. Initially a w/o emulsion is formed but, as further aqueous phase is added the emulsion inverts to form the intended product. This method often produces emulsions of very low mean droplet size.

Should any of the oily ingredients be of solid or semisolid consistency they must be melted before mixing. It is also essential that the aqueous phase be heated to the same temperature, to avoid premature solidification of the oil phase by the colder water on mixing but before emulsification has taken place. This also has the advantage of reducing the viscosity of the system, so enabling shear forces to be transmitted through the product more easily. Because of the increased kinetic motion of the emulgent molecules at the oil/water interface, however, it is necessary to continue stirring during the cooling process to avoid demulsification.

Volatile ingredients, including flavours and perfumes, are usually added after the emulsion has cooled. It must, however, be sufficiently fluid to enable adequate blending. Ingredients that may influence the physical stability of the emulsion, such as alcoholic solutions or electrolytes, require to be diluted as much as possible before adding slowly and with constant mixing.

RELEASE OF DRUGS FROM SUSPENSION AND EMULSION FORMULATIONS

Drug release from suspensions

After the oral administration of a suspension the drug, which is already in a wetted state, is presented to the gastrointestinal fluids in a finely divided form. Dissolution therefore occurs immediately. The rate of absorption of the drug into the bloodstream is therefore usually faster than for the same drug in a solid dosage form, but not as fast as that from a solution. The rate of release of a drug from a suspension is also dependent upon the viscosity of the product. The more viscous the preparation, the slower is likely to be the release of the drug. Care must therefore be taken to ensure that the physical characteristics of the suspension do not change on addition to an acid medium, if this should affect the rate of release of the drug.

Because the rate of release of an active agent from a suspension is usually slower than the release from solution, drugs are often formulated as suspensions for intramuscular, intraarticular or subcutaneous injection in order to prolong drug release. This is often termed depot therapy. Methylprednisolone, for example, which is available as the water-soluble sodium succinate salt, can be synthesized as the insoluble acetate ester. After intramuscular injection as a suspension, the rate of release is sufficiently slowed to maintain adequate blood levels for up to 14 days.

Release will occur even more slowly if the drug is suspended in an oil, which after injection will remain as a globule, so providing a minimal area of contact with tissue fluid.

Sustained release preparations formulated as suspensions for oral use are less common, but one example is the use of the Pennkinetic system. This involves the complexation of drugs such as hydrocortone and dextromethorphan with tiny ion exchange resin particles, which are then coated with ethylcellulose (Chang 1992). After ingestion the drug is slowly released by exchanging with ions present in the gastrointestinal tract. One of the main difficulties in the formulation of this type of product is to ensure that ions are not present in any of its ingredients.

Drug release from emulsions

The main commercial use of emulsions is for the oral, rectal and topical administration of oils and oil-soluble drugs. Lipid emulsions are also widely used for intravenous feeding, although the choice of emulgent is very limited and globule size must be kept below 4 μm diameter to avoid the formation of emboli. Quite often, however, the high surface area of dispersed oil globules will enhance the rate of absorption of lipophilic drugs.

The emulsion can also be used as a sustained-release dosage form. The intramuscular injection of certain water-soluble vaccines formulated as w/o emulsions can provide a slow release of the antigen

and result in a greater antibody response and hence a longer-lasting immunity. Other drugs have also been shown to have this effect, the rate of release being dependent mainly upon the oil/water partition coefficient of the drug and its rate of diffusion across the oil phase.

It is also possible to formulate multiple emulsion systems in which an aqueous phase is dispersed in oil droplets, which in turn are dispersed throughout another aqueous external phase, producing a water-in-oil-in-water (w/o/w) emulsion. These products can also be used for the prolonged release of drugs that are incorporated into the internal aqueous phase. These products have the advantage of exhibiting a lower viscosity than their w/o counterparts and hence are easier to inject.

Similarly, o/w/o emulsions can be formulated and are also under investigation as potential sustained-release bases.

Multiple emulsions, however, tend to be stable only for a relatively short time, although the use of polymers as alternatives to the traditional emulsifying agents may improve their physical stability (Florence and Whitehill 1982).

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35
Delivery of pharmaceutical proteins

Daan Crommelin, Ewoud van Winden, Albert Mekking

CHAPTER CONTENTS

INTRODUCTION

Protein structures

Pharmaceutical proteins are built up of amino acids chains (their primary structure). To be pharmacologically active, this amino acid sequence must form a well defined three-dimensional structure. Parts of the protein will fold in locally identifiable, discrete structures such as α helices or β sheets (known as secondary structures). The overall (tertiary) structure of the protein is established by the proper positioning of the different subunits relative to each other. In some cases individual protein molecules form a quaternary structure, in which the individual protein molecules interact and build a larger, well defined structure (e.g. haemoglobin).

The formation and stability of the secondary, tertiary and quaternary structures is based on relatively weak physical interactions (e.g. electrostatic interactions, hydrogen bonding, van der Waals forces and hydrophobic interactions) and not on covalent chemical-binding principles. Repulsive energy between apolar parts of the protein and water are responsible for hydrophobic interactions. The physical forces involved are relatively weak. This means that protein structures can be rather easily changed, leading to a modification or even loss of their pharmacological characteristics.

Amino acid chains can be modified by covalently attaching non-amino acid sections, such as sugar (glycoproteins), phosphate or sulphate groups. In particular, the sugar part can make up a substantial part of the molecular weight of the (glyco)protein. These groups may be essential for the pharmacological effect of a therapeutic protein, not only while acting at its receptor sites, but also to provide the proper pharmacokinetic profile.

Pharmaceutical protein molecules are large and diffusional transport through epithelial barriers such

as those encountered in the gastrointestinal tract is slow unless specific transporter molecules are available. Moreover, the conditions in the lumen of the GI tract are extremely hostile to these proteins. (Enzymatic) degradation is fast. Therefore, the large majority of pharmaceutical proteins are delivered via the parenteral route (i.e. by the needle). The issue of alternative routes of administration is discussed later in this chapter.

Conserving the integrity of these large molecules is essential to ensure an optimal therapeutic effect and to minimize effects such as the induction of unwanted immune responses. An immune response may neutralize the therapeutic activity in chronic dosing schedules and cause serious side-effects. Protein stability concerns both their chemical and their physical structure. There are many functional groups in the amino acid chain available for chemical degradation, and the preferred three-dimensional structure is readily irreversibly disturbed (e.g. through heat, changes in pH or ionic strength). Some analytical approaches to monitor the protein structure are discussed later.

The preferred shelf-life for pharmaceutical products is a minimum of 2 years. Most proteins degrade too fast when formulated as aqueous solutions, even when kept in the refrigerator. Therefore, they have to be stored in a dry form and be reconstituted before administration. These delicate structures are usually dried by freeze-drying (see Chapter 26). The choice of the proper excipients (e.g. lyoprotectants) has proved to be extremely important.

Sources of pharmaceutical proteins

Nowadays most proteins used in therapy or under development are produced by recombinant DNA or hybridoma technology (known as biotechnology or biotech products). Examples are human insulin, erythropoietin, monoclonal antibodies, cytokines and interferons. They are all produced in cell cultures by prokaryotic or eukaryotic cells, ranging from *Escherichia coli* to mammalian cells such as Chinese hamster ovary cells, or transgenic animals. From the examples listed, one may conclude that many of the pharmaceutical proteins are basically endogenous products. However, a number of currently used biotech products are not exactly identical to the endogenous product. For example, the glycosylation patterns of the recombinant form may be reproducibly produced on a large scale, but not completely match the endogenous product. Extensive evaluation of these products in clinical trials has proved their efficacy and safety.

Isolation of the expressed protein from the culture medium is a multistep process consisting of several different (chromatographic/filtration) steps. For every protein a 'tailor-made' purification protocol has to be developed to remove impurities while ensuring integrity.

Biotech-derived molecules may make up the majority of protein drugs, but there are still proteins of major therapeutic importance isolated from blood from humans or animals. Examples are albumin, blood clotting factors (such as Factor VIII from blood from human volunteers), and antisera from patients or animals such as horses and sheep. Here again, special purification protocols have to be developed, with particular emphasis on reduction of viral contamination (see later).

Specific challenges

It is clear that pharmaceutical proteins offer special challenges to the pharmaceutical formulator. They are delicate, large molecules with many functional groups. Their structure, being stabilized by relatively weak physical bonds, is readily and irreversibly changed. In vivo this may directly affect the interaction with the receptor, change their pharmacokinetic characteristics, e.g. their clearance, or make them immunogenic. Moreover, their epithelial penetration capability is very low unless the proper transporter molecules are available. Thus, as a rule, pharmaceutical proteins are administered parenterally.

In the sections that follow several issues will be dealt with in more detail.

FORMULATION OF PHARMACEUTICAL PROTEINS FOR PARENTERAL ADMINISTRATION

Stability issues

In the introduction to this chapter it was pointed out that pharmaceutical proteins are high molecular weight molecules with amino acid building blocks that are sensitive to degradation and with a specific three-dimensional structure. Table 35.1 lists the pathways for degradation of proteins.

Physical instability

Degradation rates depend on environmental conditions and the formulator should carefully select conditions for optimal stability. For example,

Table 35.1 Pathways of degradation of proteins.

Fragmentation

H_2N — Lys — Ala — COOH → H_2N — Lys + Ala — COOH

Isomerization

L-form amino acid → D-form amino acid

Deamidation

H_2N ..... Asn ..... COOH → H_2N ..... Asp ..... COOH + $—NH_2$

Oxidation

H_2N ..... Met ..... COOH → H_2N ..... Met(=O) ..... COOH

Disulphide scrambling

H_2N ..... Cys ..... Cys ..... Cys ..... COOH ⟷ H_2N ..... Cys ..... Cys ..... Cys ..... COOH

H_2N ..... Cys ..... Cys ..... Cys ..... COOH

Oligomerization

n(H_2N ..... Cys ..... Cys ..... COOH) → n(H_2N ..... Cys ..... Cys ..... COOH)

n(H_2N ..... Cys ..... Cys ..... COOH)

Aggregation

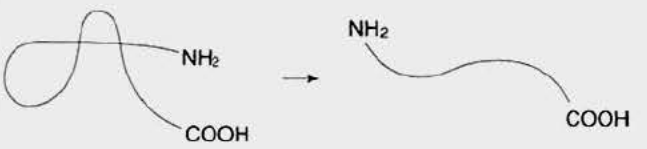
n(H_2N ..... COOH) → (H_2N ..... COOH)$_n$

Cross-linking

n(H_2N ..... COOH) → H_2N ..... COOH

H_2N ..... COOH

Denaturation



elevated temperatures can cause denaturation of proteins in aqueous solution. Interestingly, low temperatures may also induce destabilization. Besides, protein aggregation is often initiated by adsorption of the protein monomer on the walls of the container. Proteins may also aggregate by shaking or by exposure to shear forces. Hydrophobic parts of the molecule are then exposed to hydrophobic interfaces (air/water), the protein unfolds and aggregation occurs.

Chemical instability

Because of the many amino acids involved, full prevention of all chemical degradation reactions is difficult. The formulator should consider which chemical degradation pathways are relevant. Under neutral conditions the peptide bonds between amino acids are stable; only the asparagine–glycine and asparagine–proline bonds are relatively labile.

Deamidation is a rather common degradation reaction in water. Asparagine and glutamine are the amino acids that can be deamidated. Deamidation reaction kinetics depend on pH and neighbouring amino acids.

Oxidation is not limited to methionine and cysteine (Table 35.1): histidine, tryptophan and tyrosine are also sensitive to oxidation reactions. Oxidation is catalysed by traces of transition metal ions. An oxidative milieu may also cause free cysteine units to form disulphide bridges or disulphide bond scrambling.

Naturally occurring amino acids are in the L form. Isomerization to the D form is possible and will change the structure of the protein.

Improper choice of excipients may also cause degradation reactions. For example, sugars are often used as excipients (Table 35.2), but reducing sugars can react with free primary amino groups of the protein molecule via the so-called Maillard reaction (even in the dry state) and form brownish reaction products. Reducing sugars (e.g. lactose) should therefore be excluded from protein-containing formulations.

Excipients used

Table 35.2 lists the excipients used in protein-containing parenteral dosage forms. Not all of the ingredients listed are always needed, e.g. many pharmaceutical proteins are sufficiently soluble in water. This is in particular true for highly glycosylated molecules. In this case no solubility-enhancing substances are needed. However, if solubility enhancement is necessary, the selection of the proper pH conditions should first be considered. Protein solubility depends on its net charge. In general, as with low molecular weight drugs, uncharged protein molecules (at the pH of their isoelectric point, i.e.p.) have the lowest solubility in water. Therefore, choosing pH conditions 'away' from the i.e.p. can solve the protein (and the problem). Some amino acids (e.g. arginine and lysine) increase protein solubility and reduce aggregation reactions by a not-well understood mechanism. Detergents such as polysorbate 20 and 80 or sodium dodecyl sulphate can also be used to prevent aggregation. These compounds prevent the adsorption of proteins to interfaces (air/water and container/water) and thereby interface-induced protein unfolding. Human serum albumin has a strong tendency to adsorb to interfaces and may therefore be added to therapeutic protein formulations as an anti-aggregation agent.

Table 35.2 Excipients used in parenteral dosage forms and their function

Excipient	Function	Examples
Solubility-enhancing substances	Increase solubility of proteins	Amino acids, detergents
Antiadsorbent/aggregation blockers	Reduction of adsorption and aggregation prevention	Albumin, detergents
Buffer components	Stabilizing pH	Phosphate, citrate
Preservatives	Growth inhibition in vials for multiple dosing	Phenol, benzylalcohol, organic Hg-compounds
Antioxidants	Prevent oxidation	Ascorbic acid, sulphites, cysteine
Stabilizers during storage (lyoprotectants)	Preservation of integrity while in dry form	Sugars
Osmotic compounds	Ensure isotonicity	Sugars, NaCl

Oxidation reactions are catalysed by heavy metals. Chelating agents are used to reduce oxidation damage through binding of the ions. This approach cannot be used if the metal ion is necessary as an integral part of the protein structure. Examples are zinc ions in insulin formulations and iron ions in haemoglobin. Then, antioxidants such as sulphites may be added to reduce the oxidation tendency. In the case of vials for multiple dosing, preservatives have to be included in the formulation. Benzyl alcohol and phenol are often used for this purpose.

Buffered aqueous protein solutions may be stable for 2 years under refrigerator conditions. Some monoclonal antibody formulations, for example, are available as aqueous solutions, but the more common situation is that the formulation has to be freeze-dried in the vials to avoid degradation and to ensure that the product can be readily reconstituted.

During freeze-drying (Chapter 26) water is removed by sublimation. In the freeze-drying process three discrete phases can be discerned. The first is freezing of the solution to temperatures typically around −35 to −40°C, followed by a sublimation phase with temperatures of around −35°C and low pressures to remove the frozen water (phase 2), and a final, secondary drying stage to remove most residual water. The pressure must remain low, but the temperature can rise up to about 20°C without collapse of the porous cake (see below). A lyoprotectant (e.g. sugar) is necessary to stabilize the product as the removal of water may irreversibly affect the protein structure. Moreover, sugar lyoprotectants also happen to form readily reconstitutable porous cakes.

The freezing temperature should be low enough to convert the aqueous solution with the sugar and the protein into a glass. Glass formation in sugar solutions usually occurs around −30°C. Just below the glass transition temperature the sublimation process can begin during the lowering of the pressure in the chamber. The sublimated water is collected on a condenser with a considerably lower temperature (typically −60°C). As sublimation extracts a large amount of latent heat from the system, the temperature in the vials containing the frozen protein solution could fall even lower than the starting temperature, slowing down sublimation. The vials are therefore heated in a controlled way to keep them at temperatures low enough to preserve their glassy texture, but high enough to let the sublimation process proceed at a sufficiently high speed.

The mechanism(s) of action of lyoprotectants (non-reducing sugars) are not fully understood. The following may play a role:

- Lyoprotectants replace water as stabilizing agent ('water replacement theory') of the protein;
- Lyoprotectants increase the glass transition temperature in the frozen system and in the dried system, avoiding collapse of the porous cake which would slow down water removal from the frozen cake (during freeze drying) and interfere with a rapid reconstitution of the freeze-dried cake;
- Lyoprotectants slow down the secondary drying process and minimize the chances of overdrying of the product in the secondary drying stage.

Microbiological requirements

Typically, pharmaceutical proteins are administered via the parenteral route. This implies that the product should be sterile. In addition, virus and pyrogen removal steps should be part of the purification and production protocol.

Pharmaceutical proteins cannot be sterilized by autoclaving, gas sterilization or ionizing radiation, because these procedures damage the molecules. Therefore, sterilization of the end-product is not possible. This leaves aseptic manufacturing as the only option.

All utensils and components must be presterilized (by heat sterilization, ionizing radiation or membrane filtration) before assembling the final formulation to minimize the bioburden. Protein products are manufactured under aseptic conditions in class 100 areas (fewer than 100 particles > 0.5 μm per cubic foot). This low level contamination is reached by filtration of air through HEPA (high-efficiency particulate air) filters. Finally, the product is filled into the containers through sterile filters with 0.22 μm pores before capping or freeze drying/capping.

Pharmaceutical proteins are produced by living organisms. Viruses can be introduced into the product either by the use of contaminated culture media or via infected (mammalian) production cells. It is therefore important that purification and manufacturing protocols contain viral decontamination steps. Viral decontamination can be accomplished by virus removal and/or by viral inactivation. The problem faced when selecting inactivation techniques is that there is often a narrow window between successful viral inactivation and preservation of the integrity of the pharmaceutical protein structure.

Viruses can be removed by filtration, precipitation or chromatography. For virus inactivation, heat treatment (pasteurization), radiation or crosslinking agents (e.g. β-propiolactone) can be used. As no single process guarantees complete virus removal, often

several different decontamination steps are introduced in series in the 'downstream' purification process and in the manufacturing of the final formulation.

Gram-negative host cells, such as *E. coli*, are often used as production cells for non-glycosylated proteins. Gram-negative cells contain large amounts of endotoxins in their membranes. These endotoxins are heat stable, amphipatic, negatively charged lipopolysaccharides and are potent pyrogens. Pyrogens have to be removed in order to meet pharmacopoeial criteria, and this can be done, for example, through anion-exchange chromatography.

ANALYTICAL TECHNIQUES TO CHARACTERIZE PROTEINS

It is clearly important to be able to guarantee the integrity of a protein. As mentioned earlier, a protein molecule is a complex three-dimensional structure of amino acids, often coupled to saccharide, phosphate or sulphate moieties. The total structure is responsible for the pharmacodynamic (e.g. receptor interaction) and pharmacokinetic (e.g. clearance, targeting) effect. It is not possible to define the structure of a pharmaceutical protein with the same precision as small, low molecular weight molecules, where a combination of analytical techniques provides unequivocal structural evidence.

Therefore, a set of pharmacological, immunological, spectroscopic, electrophoretic and chromatographic approaches is used to characterize the protein as closely as possible. Table 35.3 lists a number of regularly used analytical techniques and the information that is obtained.

Quality assessment used to be based on functional tests in vivo (relevant animal models). An example is the pharmacopoeial test for insulin: the lowering of the blood glucose level in rabbits upon

Table 35.3 Approaches to confirm protein structure

Approach	Information obtained
In vivo tests, use of test animals	Pharmacological effect
In vitro tests (sensitive cells)	Functional test
Immunological tests	
ELISA	Interaction with one epitope on protein
RIA	Interaction with one epitope on protein
Analytical approaches	
Spectroscopic	
UV spectroscopy	Secondary/tertiary structure
fluorimetry	Secondary/tertiary structure
CD spectroscopy	Secondary/tertiary structure
infrared spectroscopy	Secondary/tertiary structure
mass spectrometry	Secondary/tertiary structure
Electrophoretic approaches	
SDS-PAGE	Molecular weight
IEF	Isoelectric point
High-performance liquid chromatography (HPLC)	
GP (gel permeation)	Molecular weight/aggregates
HI (hydrophobic interaction)	Hydrophobic interactions
Affinity chromatography	Interaction with specific ligand
IEC (ion exchange)	Charge patterns
RP (reversed phase)	

CD, circular dichroism;
ELISA, enzyme-linked immunosorbent assay;
IEC, ion-exchange chromatography;
IEF, isoelectric focusing;
HPLC, high-performance liquid chromatography;
MALDI, matrix-assisted laser desorption ionization;
MS, mass spectrometry;
RIA, radioimmunoassay;
SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis;

injection of the insulin product to be tested. These tests do not have the sensitivity to identify small changes in molecular structure or detect early degradation products, and they do not provide information on such things as the presence of product immunogenicity. In vitro cell tests, such as those used for cytokine activity assessment, inform us about the functional activity of the molecule, but not its pharmacokinetic behaviour or immunogenicity. ELISA (enzyme-linked immunosorbent assay) and RIA (radioimmunoassay) belong to the class of immunological tests. Here the interaction of a monoclonal antibody with one epitope region on the protein is determined. The rest of the molecule is not 'probed'.

Electrophoretic techniques such as SDS-PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis) and IEF (isoelectric focusing) are powerful tools to assess product purity and to provide molecular weight and isoelectric point (i.e.p.) information regarding the protein.

Table 35.3 lists a number of chromatographic techniques that elucidate product characteristics. In particular, impurities and degradation products can be picked up at an early stage. Gel chromatography discriminates mainly on the basis of molecular size and is a powerful technique to monitor aggregate formation. Ion-exchange resins separate on the basis of subtle variations in protein charge patterns and are being used to detect oxidation (e.g. methionine) and deamidated (converted glutamine and asparagine) products. Modern mass spectroscopic techniques such as MALDI–TOF (matrix-assisted laser desorption ionization–time of flight) mass spectroscopic analysis, or a combination of HPLC (high-pressure liquid chromatography) with electrospray ionization-induced mass spectrometry give detailed information on amino acid sequence and glycosylation patterns.

In conclusion, to ensure pharmaceutical protein quality one must follow a strict protocol regarding the definition of the protein production cell lines used, the chosen culturing conditions and downstream processing conditions, and the filling/(drying)/ finishing process. Analytical approaches to confirm the protein structure will always include a long list of approaches, ranging from in vivo tests in animals to information provided by highly sophisticated analytical technologies.

None of these tests tells the whole story; together they tell more, but there is never the situation encountered with many low molecular weight molecules whereby a full description of the drug, including a detailed impurity profile, is available.

ADMINISTRATION OF PHARMACEUTICAL PROTEINS

Routes of administration

As mentioned in the introduction to this chapter, oral administration of a pharmaceutical protein results in a very low bioavailability. The protein is enzymatically attacked in the gastrointestinal tract and, moreover, penetration through the gut wall will be slow and incomplete. Oral vaccines containing antigenic protein material are an exception to the general rule that proteins should not be administered orally. With vaccines, even low uptake levels may still deliver sufficient material to lymphoid tissue just below the epithelium (in the so-called Peyer's patches) to induce a strong (both local and systemic) immune response.

When a protein is delivered intravenously clearance from the blood compartment can be fast, with a half-life of minutes, or slow, with a half-life of several days. An example of a rapidly cleared protein is tissue plasminogen activiator (tPA), with a plasma half-life of a few minutes. On the other hand, human monoclonal antibodies have half-lives of the order of days.

Protein drugs are often administered subcutaneously or intramuscularly. These routes of administration are considered to be more patient friendly and the injection process easier than with the intravenous route. Upon intramuscular (i.m.) or subcutaneous (s.c.) injection the protein is not instantaneously drained to the blood compartment. Studies monitoring the fate of a protein upon s.c. injection demonstrate that passage of a protein through the endothelial barrier lining the local capillaries at the site

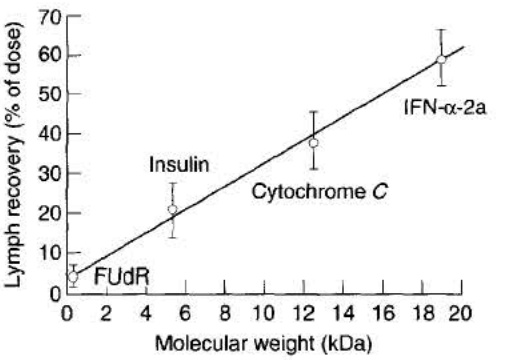


Fig. 35.1 Correlation between the molecular weight and the cumulative recovery of recombinant interferon (IFN α-2a), cytochrome C, inulin and 5-Fluoro-2'-deoxyuridine (FudR) in the efferent lymph from the right popliteal lymph node following s.c. administration into the lower part of the right hind leg of sheep (from Crommelin and Sindelar, 1998)

of injection is size dependent. If the protein is too large it will enter the lymphatic system and be transported via the lymph into the blood. Figure 35.1 shows the relationship between molecular size and lymphatic drainage. Lymphatic drainage takes time and a delay in the onset of systemic activity is observed. The protein is also exposed to the local environment containing proteases. Therefore, the bioavailability of protein drugs upon s.c. (and i.m.) administration can be far from 100%. This can have dramatic consequences, e.g. some diabetics become insulin resistant because of high tissue peptidase activity.

There is not always a direct relationship between plasma level and pharmacological response (i.e. no direct pharmacokinetic–pharmacodynamic (PK/PD) relationship). As the mechanism of action of a drug might be complex, involving different sequential steps, fast clearance from the blood compartment may not necessarily mean that drug action is also short-lived. The relationship between a pharmacokinetic profile and the pharmacodynamic result of the presence of the drug can be quite complex. A drug may trigger a reaction, which may result in measurable, pharmacological effects much later. As an example, the cytokine intraleukin-2 (IL-2) (in its PEG-ylated form) is rapidly cleared from the blood compartment and a pharmacological effect (increase in the number of blood lymphocytes) is observed long afterwards (can be days) (Fig. 35.2).

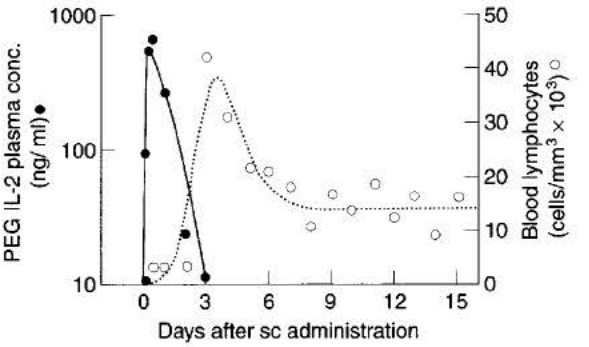


Fig. 35.2 PEG-IL-2 pharmacokinetics and pharmacodynamics (changes in blood lymphocyte count) after subcutaneous adminstration of 10 MIU/kg in rats. PEG = poly(ethylene glycol); (from Crommelin and Sindelar 1998)

Finding alternatives for the parenteral route has been an area of interest for many years. Table 35.4 lists different possible routes of delivery for proteins.

With the exception of the pulmonary route, all other options have a low bioavailability. Some bioavailability data on the intratracheal administration of proteins in rats are shown in Table 35.5. The extent of absorption depends strongly on the nature

Table 35.4 Alternative routes of administration to the oral route for biopharmaceuticals (adapted from Crommelin and Sindelar 1998)

Route	Relative advantage	Relative disadvantage
Nasal	Easily accessible, fast uptake, proven track record with a number of 'conventional' drugs, probably lower proteolytic activity than in the GI tract, avoidance of first-pass effect, spatial containment of absorption enhancers is possible	Reproducibility (in particular under pathological conditions), safety (e.g. ciliary movement), low bioavailability for proteins
Pulmonary	Relatively easy to access, fast uptake, proven track record with 'conventional' drugs, substantial fractions of insulin are absorbed, lower proteolytic activity than in the GI tract, avoidance of hepatic first-pass effect, spatial containment of absorption enhancers (?)	Reproducibility (in particular under pathological conditions, smokers/non-smokers), safety (e.g. immunogenicity), presence of macrophages in the lung with high affinity for particulates
Rectal	Easily accessible, partial avoidance of hepatic first-pass effect, probably lower proteolytic activity than in the upper parts of the GI tract, spatial containment of absorption enhancers is possible, proven track record with a number of 'conventional' drugs	Low bioavailability for proteins
Buccal	Easily accessible, avoidance of hepatic first-pass effect, probably lower proteolytic activity than in the lower parts of the GI tract, spatial containment of absorption enhancers is possible, option to remove formulation if necessary	Low bioavailability of proteins, no proven track record yet (?)
Transdermal	Easily accessible, avoidance of hepatic first-pass effect, removal of formulation is possible if necessary, spatial containment of absorption enhancers is possible, proven track record with 'conventional' drugs, sustain/controlled release possible	Low bioavailability of proteins

Table 35.5 Absolute bioavailability of a number of proteins (intratracheal vs intravenous) in rats

Molecule	MW (kDa)	No. of amino acids	Absolute bioavailability (%)
α-Interferon	20	165	> 56
PTH-84	9	84	> 20
PTH-34	4.2	34	40
Calcitonin (human)	3.4	32	17
Calcitonin (salmon)	3.4	32	17
Glucagon	3.4	29	< 1
Somatostatin	3.1	28	< 1

PTH, recombinant human parathyroid hormone.
(From Crommelin and Sindelar 1998 with permission)

of the protein. Insulin is a candidate drug for pulmonary delivery to diabetics to mimic the natural physiological response to a meal (postprandial glucose control). Subcutaneous injection gives a relatively slow response; pulmonary uptake is faster. New pulmonary delivery devices (see Chapter 31) not only increase average bioavailability but also reduce variation in uptake.

Three approaches have been followed to improve the bioavailability of pharmaceutical proteins when exploring alternative routes of administration. First, coadministration of protease inhibitors, such as bacitracin, should slow down metabolic degradation. Second, excipients (often with an amphipatic character, such as bile salts) can be added to enhance passage through epithelial barriers. The third approach is to prolong the presence of the protein at the absorption surface, e.g. by the use of mucoadhesives.

Intranasal delivery of chitosan and starch microspheres demonstrated enhanced uptake of coadministered insulin. In humans, intranasal delivery of insulin with chitosan results in absolute bioavailabilities of 7%. In conclusion, bioavailability is indeed strongly enhanced when using these approaches, but safety issues must be addressed before these absorption enhancers can be introduced into marketed products.

Release control

Many therapeutic proteins are short-lived in the blood compartment. Assuming there is a direct relationship between blood level and therapeutic effect, it is important to maintain therapeutically relevant drug concentrations in the bloodstream. Portable pump systems with adjustable pump rates are available for patients. Catheters provide the link between the pump and, for example, the peritoneal cavity. These systems are particularly useful if a constant dose input is required and the drug is needed over a limited period of time. Otherwise, more flexible delivery systems are preferred. For insulin (with a plasma half-life of 5 minutes) different forms of controlled-release systems for s.c. injection are available. Release control is based on different physicochemical appearances (amorphous/crystalline) of insulin itself and on insulin complexes with Zn^{2+} ions or proteins, such as protamine. Zn^{2+} ions tend to slow down the release of insulin. Amorphous insulin plus Zn^{2+} ions results in moderate prolongation of drug action. Crystalline insulin plus Zn^{2+} ions gives a long-acting product. The addition of protamine (at neutral pH a positively charged protein) to insulin and Zn^{2+} ion combinations protracts the insulin effects even more (up to 72 hours; long-acting). Isophane insulin (NPH: neutral protamine Hagedorn) contains insulin and protamine in isophane proportions (no excess of either component), resulting in intermediate-acting formulations.

At present, efforts are being made to build 'closed-loop' systems where insulin administration is controlled by:

- a biosensor permanently monitoring blood glucose levels;
- an infusion pump with adjustable pump rate; and
- an electronic section with an algorithm linking blood glucose levels to insulin need at any time.

In hospitals such equipment is available to stabilize blood glucose in patients for limited periods, but no portable 'patient-friendly' systems for chronic use are yet available.

In even earlier stages of development are 'artificial pancreases'. Isolated insulin-producing β cells from the islets of Langerhans are introduced into the body in a container system with a wall that allows the passage of glucose, insulin and nutrients. However, the container wall keeps the β cells separated from the patient's immune system. Increasing blood glucose levels will stimulate the secretion of insulin by the encapsulated β cells. The excreted insulin will be released from the container and glucose levels will fall until normal blood levels are reached.

Controlled-release systems containing microspheres (with diameters between 10 and 100 μm) for s.c. administration are currently under development. A 30-day action sustained-release system designed on the basis of biodegradable polylactic-glycolic acid

has been formulated for human growth hormone. These microspheres are prepared using a double emulsion technique whereby the proteins are exposed to organic solvents, and protein encapsulation efficiency is rather low. Alternatively, a dextran-based microsphere preparation protocol has been developed without the use of organic solvents and with extremely high loading efficiencies.

CONCLUDING REMARKS

Protein drugs are rapidly gaining in importance, and both market volume and market share are expected to rise. Biotechnological techniques permit the design and synthesis of active proteins. It is the task of the pharmaceutical formulation scientist to turn the pure substance into a formulation that can be safely administered to the patient, exerting optimal therapeutic benefits. In this chapter different aspects of this formulation process are described and special attention is given to those aspects where biotech products clearly differ from low molecular weight drugs.

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