CHAPTER 1

COMMON PHYSICAL TECHNIQUES USED IN PURIFICATION

INTRODUCTION

Purity is a matter of degree. Other than adventitious contaminants such as dust, paper fibres, wax, cork, etc., that may have been incorporated into the sample during manufacture, all commercially available chemical substances are in some measure impure. Any amounts of unreacted starting material, intermediates, by-products, isomers and related compounds may be present depending on the synthetic or isolation procedures used for preparing the substances. Inorganic reagents may deteriorate because of defective packaging (glued liners affected by sulfuric acid, zinc extracted from white rubber stoppers by ammonia), corrosion or prolonged storage. Organic molecules may undergo changes on storage. In extreme cases the container may be incorrectly labelled or, where compositions are given, they may be misleading or inaccurate for the proposed use. Where any doubt exists it is usual to check for impurities by appropriate spot tests, or by recourse to tables of physical or spectral properties such as the extensive infrared and NMR libraries published by the Sigma Aldrich Chemical Co.
The important question, then, is not whether a substance is pure but whether a given sample is sufficiently pure for some intended purpose. That is, are the contaminants likely to interfere in the process or measurement that is to be studied. By suitable manipulation it is often possible to reduce levels of impurities to acceptable limits, but absolute purity is an ideal which, no matter how closely approached, can never be attained. A *negative* physical or chemical test indicates only that the amount of an impurity in a substance lies below a certain sensitivity level; no test can demonstrate that a specified impurity is entirely absent.

When setting out to purify a laboratory chemical, it is desirable that the starting material is of the best grade commercially available. Particularly among organic solvents there is a range of qualities varying from *laboratory chemical* to *spectroscopic* and *chromatographic* grades. Many of these are suitable for use as received. With many of the more common reagents it is possible to obtain from the current literature some indications of likely impurities, their probable concentrations and methods for detecting them. However, in many cases complete analyses are not given so that significant concentrations of unspecified impurities may be present.

THE QUESTION OF PURITY

Solvents and substances that are specified as *pure* for a particular purpose may, in fact, be quite impure for other uses. Absolute ethanol may contain traces of benzene, which makes it unsuitable for ultraviolet spectroscopy, or plasticizers which make it unsuitable for use in solvent extraction.

Irrespective of the grade of material to be purified, it is essential that some criteria exist for assessing the degree of purity of the final product. The more common of these include:

1. Examination of physical properties such as:
   (a) Melting point, freezing point, boiling point, and the freezing curve (i.e. the variation, with time, in the freezing point of a substance that is being slowly and continuously frozen).
   (b) Density.
   (c) Refractive index at a specified temperature and wavelength. The sodium D line at 589.26 nm (weighted mean of D_1 and D_2 lines) is the usual standard of wavelength but results from other wavelengths can often be interpolated from a plot of refractive index versus $1/(\text{wavelength})^2$.

(d) Specific conductivity. (This can be used to detect, for example, water, salts, inorganic and organic acids and bases, in non-electrolytes).
(e) Optical rotation, optical rotatory dispersion and circular dichroism.

2. Empirical analysis, for C, H, N, ash, etc.

3. Chemical tests for particular types of impurities, e.g. for peroxides in aliphatic ethers (with acidified KI), or for water in solvents (quantitatively by the Karl Fischer method, see Fieser and Fieser, Reagents for Organic Synthesis J. Wiley & Sons, NY, Vol 1 pp. 353, **528**, *1967*, Library of Congress Catalog Card No 66-27894).

4. Physical tests for particular types of impurities:
   (a) Emission and atomic absorption spectroscopy for detecting organic impurities and determining metal ions.
   (b) Chromatography, including paper, thin layer, liquid (high, medium and normal pressure) and vapour phase.
   (c) Electron spin resonance for detecting free radicals.
   (d) X-ray spectroscopy.
   (e) Mass spectroscopy.
   (f) Fluorimetry.

5. Examination of spectroscopic properties
   (a) Nuclear Magnetic Resonance (^{1}H, ^{13}C, ^{31}P, ^{19}F NMR etc)
   (b) Infrared spectroscopy (IR)
   (c) Ultraviolet spectroscopy (UV)
   (d) Mass spectroscopy [electron ionisation (EI), electron ionisation (CI), electrospray ionisation (ESI), fast atom bombardment (FAB), matrix-associated laser desorption ionisation (MALDI), etc]

6. Electrochemical methods (see Chapter 6 for macromolecules).

7. Nuclear methods which include a variety of radioactive elements as in organic reagents, complexes or salts.

A substance is usually taken to be of an acceptable purity when the measured property is unchanged by further treatment (especially if it agrees with a recorded value). In general, at least two different methods, such as recrystallisation and distillation, should be used in order to ensure maximum purity. Crystallisation may be repeated (from the same solvent or better from different solvents) until the substance has a constant melting point or absorption spectrum, and until it distils repeatedly within a narrow, specified temperature range.

With liquids, the refractive index at a specified temperature and wavelength is a sensitive test of purity. Note however that this is sensitive to dissolved gases such as O_2, N_2 or CO_2. Under favourable conditions, freezing curve studies are sensitive to impurity levels of as little as 0.001 moles per cent. Analogous fusion curves or heat capacity measurements can be up to ten times as sensitive as this. With these exceptions, most of the above methods are rather insensitive, especially if the impurities and the substances in which they occur are chemically similar. In some cases, even an impurity comprising many parts per million of a sample may escape detection.

The common methods of purification, discussed below, comprise distillation (including fractional distillation, distillation under reduced pressure, sublimation and steam distillation), crystallisation, extraction, chromatographic and other methods. In some cases, volatile and other impurities can be removed simply by heating. Impurities can also sometimes be eliminated by the formation of derivatives from which the purified material is regenerated (see Chapter 2).

SOURCES OF IMPURITIES

Some of the more obvious sources of contamination of solvents arise from storage in metal drums and plastic containers, and from contact with grease and screw caps. Many solvents contain water. Others have traces of acidic materials such as hydrochloric acid in chloroform. In both cases this leads to corrosion of the drum and contamination of the solvent by traces of metal ions, especially Fe^{3+}. Grease, for example on stopcocks of separating funnels and other apparatus, e.g. greased ground joints, is also likely to contaminate solvents during extractions and chemical manipulation.

A much more general source of contamination that has not received the consideration it merits comes from the use of plastics for tubing and containers. Plasticisers can readily be extracted by organic solvents from PVC and other plastics, so that most solvents, irrespective of their grade (including spectrograde and ultrapure) have been reported to contain 0.1 to 5ppm of plasticiser [de Zeeuw, Jonkman and van Mansvelt *Anal Biochem* **67** 339 *1975*]. Where large quantities of solvent are used for extraction (particularly of small amounts of compounds), followed by evaporation, this can introduce significant amounts of impurity, even exceeding the weight of the genuine extract and giving rise to spurious peaks in gas chromatography (for example of fatty acid methyl esters [Pascaud, *Anal Biochem* **18** 570 *1967*]. Likely contaminants are di(2-ethylhexyl)phthalate and dibutyl phthalate, but upwards of 20 different phthalate esters are listed as plasticisers as well as adipates, azelates, phosphates, epoxides, polyesters and various heterocyclic compounds. These plasticisers would enter the solvent during passage through plastic tubing or from storage in containers or from plastic coatings used in cap liners for bottles. Such contamination could arise at any point in the manufacture or distribution of a solvent. The problem with cap liners is avoidable by using corks wrapped in aluminium foil, although even in this case care should be taken because aluminium foil can dissolve in some liquids e.g. benzylamine and propionic acid.
Solutions in contact with polyvinyl chloride can become contaminated with trace amounts of lead, titanium, tin, zinc, iron, magnesium or cadmium from additives used in the manufacture and moulding of PVC.
N-Phenyl-2-naphthylamine is a contaminant of solvents and biological materials that have been in contact with black rubber or neoprene (in which it is used as an antioxidant). Although it was only an artefact of the separation procedure it has been isolated as an apparent component of vitamin K preparations, extracts of plant lipids, algae, livers, butter, eye tissue and kidney tissue [Brown *Chem Br* **3** 524 *1967*].
Most of the above impurities can be removed by prior distillation of the solvent, but care should be taken to avoid plastic or black rubber as much as possible.

PRACTICES TO AVOID IMPURITIES

Cleaning practices

Laboratory glassware and Teflon equipment can be cleaned satisfactorily for most purposes by careful immersion into a solution of sodium dichromate in concentrated sulfuric acid, followed by draining, and rinsing copiously with distilled water. This is an exothermic reaction and should be carried out **very** cautiously in an efficient fume cupboard. [To prepare the chromic acid bath, dissolve 5 g of sodium dichromate (CARE: cancer suspect agent) in 5 mL of water.The dichromate solution is then cooled and stirred while 100 mL of concentrated sulfuric acid is added slowly. Store in a glass bottle.] Where traces of chromium (adsorbed on the glass) must be avoided, a 1:1 mixture of concentrated sulfuric and nitric acid is a useful alternative. (*Use in a fumehood to remove vapour and with adequate face protection.*) Acid washing is also suitable for polyethylene ware but prolonged contact (some weeks) leads to severe deterioration of the plastic. Alternatively an alcoholic solution of sodium hydroxide (alkaline base bath) can be used. This strongly corrosive solution (CAUTION: Alkali causes serious burns) can be made by dissolving 120g of NaOH in 120 mL water, followed by dilution to 1 L with 95% ethanol. This solution is conveniently stored in suitable alkali resistant containers (e.g. Nalgene heavy duty rectangular tanks) with lids. Glassware can be soaked overnight in the base bath and rinsed thoroughly after soaking. For much glassware, washing with hot detergent solution, using tap water, followed by rinsing with distilled water and acetone, and heating to 200-300° overnight, is adequate. (Volumetric apparatus should not be heated: after washing it is rinsed with acetone, then hexane, and air-dried. Prior to use, equipment can be rinsed with acetone, then with petroleum ether or hexane, to remove the last traces of contaminants.) Teflon equipment should be soaked, first in acetone, then in petroleum ether or hexane for ten minutes prior to use.
For trace metal analyses, prolonged soaking of equipment in 1M nitric acid may be needed to remove adsorbed metal ions.
Soxhlet thimbles and filter papers may contain traces of lipid-like materials. For manipulations with highly pure materials, as in trace-pesticide analysis, thimbles and filter papers should be thoroughly extracted with hexane before use.
Trace impurities in silica gel for TLC can be removed by heating at 300° for 16h or by Soxhlet extraction for 3h with distilled chloroform, followed by 4h extraction with distilled hexane.

Silylation of glassware and plasticware

Silylation of apparatus makes it repellant to water and hydrophilic materials. It minimises loss of solute by adsorption onto the walls of the container. The glassware is placed in a desiccator containing dichloromethyl silane (1mL) in a small beaker and evacuated for 5min. The vacuum is turned off and air is introduced into the desiccator which allows the silylating agent to coat the glassware uniformly. The desiccator is then evacuated, closed and set aside for 2h. The glassware is removed from the desiccator and baked at 180° for 2h before use.

Plasticware is treated similarly except that it is rinsed well with water before use instead of baking. Note that dichloromethyl silane is highly **TOXIC** and **VOLATILE**, and the whole operation should be carried out in an efficient fume cupboard.
An alternative procedure used for large apparatus is to rinse the apparatus with a 5% solution of dichloromethyl silane in chloroform, followed by several rinses with water before baking the apparatus at 180°/2h (for glass) or drying in air (for plasticware).
Plus One REPEL-SILANE ES (a solution of 2% w/v of dichloromethyl silane in octamethyl cyclooctasilane) is used to inhibit the sticking of polyacrylamide gels, agarose gels and nucleic acids to glass surfaces and is available commercially (Amersham Biosciences).

SAFETY PRECAUTIONS ASSOCIATED WITH THE PURIFICATION OF LABORATORY CHEMICALS

Although most of the manipulations involved in purifying laboratory chemicals are inherently safe, care is necessary if hazards are to be avoided in the chemical laboratory. In particular there are dangers inherent in the inhalation of vapours and absorption of liquids and low melting solids through the skin. In addition to the toxicity of solvents there is also the risk of their flammability and the possibility of eye damage. Chemicals, particularly in admixture, may be explosive. Compounds may be carcinogenic or otherwise deleterious to health. Present day chemical catalogues specifically indicate the particular dangerous properties of the individual chemicals they list and these should be consulted whenever the use of commercially available chemicals is contemplated. Radioisotopic labelled compounds pose special problems of human exposure and of disposal of laboratory waste. Hazardous purchased chemicals are accompanied by detailed MSDS (Material Safety Data Sheets), which contain information regarding their toxicity, safety handling procedures and the necessary precautions to be taken. These should be read carefully and filed for future reference. In addition, chemical management systems such as ChemWatch which include information on hazards, handling and storage are commercially available. There are a number of websites which provide selected safety information: they include the Sigma-Aldrich website (www.sigmaaldrich.com) and other chemical websites e.g. www.ilpi.com/msds).

The most common hazards are:

(1) Explosions due to the presence of peroxides formed by aerial oxidation of ethers and tetrahydrofuran, decahydronaphthalene, acrylonitrile, styrene and related compounds.
(2) Compounds with low flash points (below room temperature). Examples are acetaldehyde, acetone, acetonitrile, benzene, carbon disulfide, cyclohexane, diethyl ether, ethyl acetate and *n*-hexane.
(3) Contact of oxidising agents ($KMnO_4$, $HClO_4$, chromic acid) with organic liquids.
(4) Toxic reactions with tissues.

The laboratory should at least be well ventilated and safety glasses should be worn, particularly during distillation and manipulations carried out under reduced pressure or elevated temperatures. With this in mind we have endeavoured to warn users of this book whenever greater than usual care is needed in handling chemicals. As a general rule, however, **all chemicals which users are unfamiliar with should be treated with extreme care and assumed to be highly flammable and toxic.** The safety of others in a laboratory should always be foremost in mind, with ample warning whenever a potentially hazardous operation is in progress. Also, unwanted solutions or solvents should never be disposed of *via* the laboratory sink. The operator should be aware of the usual means for disposal of chemicals in her/his laboratories and she/he should remove unwanted chemicals accordingly. **Organic liquids for disposal should be temporarily stored, as is practically possible, in respective containers. Avoid placing all organic liquids in the same container particularly if they contain small amounts of reagents which could react with each other. Halogenated waste solvents should be kept separate from other organic liquids.**

SOME HAZARDS OF CHEMICAL MANIPULATION IN PURIFICATION AND RECOVERY OF RESIDUES

Performing chemical manipulations calls for some practical knowledge if danger is to be avoided. However, with care, hazards can be kept to an acceptable minimum. A good general approach is to consider every operation as potentially perilous and then to adjust one's attitude as the operation proceeds. A few of the most common dangers are set out below. For a larger coverage of the following sections, and of the literature, the bibliography at the end of this chapter should be consulted.

Perchlorates and perchloric acid. At 160° perchloric acid is an exceedingly strong oxidising acid and a strong dehydrating agent. Organic perchlorates, such as methyl and ethyl perchlorates, are unstable and are violently explosive compounds. A number of heavy-metal perchlorates are extremely prone to explode. The use of anhydrous magnesium perchlorate, *Anhydrone*, *Dehydrite*, as a drying agent for organic vapours is **not** recommended. Desiccators which contain this drying agent should be adequately shielded at all times and kept in a cool place, i.e. **never** on a window sill where sunlight can fall on it.
No attempt should be made to purify perchlorates, except for ammonium, alkali metal and alkaline earth salts which, in water or aqueous alcoholic solutions are insensitive to heat or shock. Note that perchlorates react relatively slowly in aqueous organic solvents, but as the water is removed there is an increased possibility of an explosion. Perchlorates, often used in non-aqueous solvents, are explosive in the presence of even small amounts of organic compounds when heated. Hence stringent care should be taken when purifying perchlorates, and direct flame and infrared lamps should be avoided. Tetra-alkylammonium perchlorates should be dried below 50° under vacuum (and protection). Only very small amounts of such materials should be prepared, and stored, at any one time.

Peroxides. These are formed by aerial oxidation or by autoxidation of a wide range of organic compounds, including diethyl ether, allyl ethyl ether, allyl phenyl ether, dibenzyl ether, benzyl butyl ether, *n*-butyl ether, *iso*-butyl ether, *t*-butyl ether, dioxane, tetrahydrofuran, olefins, and aromatic and saturated aliphatic hydrocarbons. They accumulate during distillation and can detonate violently on evaporation or distillation when their concentration becomes high. If peroxides are likely to be present materials should be tested for peroxides before distillation (for tests see entry under "Ethers", in Chapter 2). Also, distillation should be discontinued when at least one quarter of the residue is left in the distilling flask.

Heavy-metal-containing-explosives. Ammoniacal silver nitrate, on storage or treatment, will eventually deposit the highly explosive silver nitride *"fulminating silver"*. Silver nitrate and ethanol may give silver fulminate (see Chapter 5), and in contact with azides or hydrazine and hydrazides may form silver azide. Mercury can also form such compounds. Similarly, ammonia or ammonium ions can react with gold salts to form *"fulminating gold"*. Metal fulminates of cadmium, copper, mercury and thallium are powerfully explosive, and some are detonators [Luchs, *Photog Sci Eng* **10** 334 *1966*]. Heavy metal containing solutions, particularly when organic material is present should be treated with great respect and precautions towards possible explosion should be taken.

Strong acids. In addition to perchloric acid (see above), extra care should be taken when using strong mineral acids. Although the effects of concentrated sulfuric acid are well known these cannot be stressed strongly enough. Contact with tissues will leave irreparable damage. **Always dilute the concentrated acid by carefully adding the acid down the side of the flask which contains water, and the process should be carried out under cooling. This solution is not safe to handle until the acid has been thoroughly mixed with the water. Protective face, and body coverage should be used at all times.** Fuming sulfuric acid and chlorosulfonic acid are even more dangerous than concentrated sulfuric acid and adequate precautions should be taken. Chromic acid cleaning mixture contains strong sulfuric acid and should be treated in the same way; and in addition the mixture is potentially ***carcinogenic***.
Concentrated and fuming nitric acids are also dangerous because of their severe deleterious effects on tissues.

Reactive halides and anhydrides. Substances like acid chlorides, low molecular weight anhydrides and some inorganic halides (e.g. PCl_3) can be **highly toxic and lachrymatory affecting mucous membranes and lung tissues. Utmost care should be taken when working with these materials. Work should be carried out in a very efficient fume cupboard.**

Solvents. The flammability of low-boiling organic liquids cannot be emphasised strongly enough. These invariably have very low flash points and can ignite spontaneously. Special precautions against explosive flammability should be taken when recovering such liquids. Care should be taken with small volumes (*ca* 250mL) as well as large volumes (> 1L), and the location of all the fire extinguishers, and fire blankets, in the immediate vicinity of the apparatus should be checked. The fire extinguisher should be operational. The following flammable liquids (in alphabetical order) are common fire hazards in the laboratory: acetaldehyde, acetone, acrylonitrile, acetonitrile, benzene, carbon disulfide, cyclohexane, diethyl ether, ethyl acetate, hexane, low-boiling petroleum ether, tetrahydrofuran and toluene. Toluene should always be used in place of benzene wherever possible due to the potential *carcinogenic* effects of the liquid and vapour of the latter.
The drying of flammable solvents with sodium or potassium metal and metal hydrides poses serious potential fire hazards and adequate precautions should be stressed.

Salts. In addition to the dangers of perchlorate salts, other salts such as nitrates, azides and diazo salts can be hazardous and due care should be taken when these are dried. Large quantities should never be prepared or stored for long periods.

SAFETY DISCLAIMER

Experimental chemistry is a very dangerous occupation and extreme care and adequate safety precautions should be taken at all times. Although we have stated the safety measures that have to be taken under specific entries these are by no means exhaustive and some may have been unknowingly or accidentally omitted. The experimenter without prior knowledge or experience must seek further safety advice on reagents and procedures from experts in the field before undertaking the purification of any material, We take no responsibility whatsoever if any mishaps occur when using any of the procedures described in this book.

METHODS OF PURIFICATION OF REAGENTS AND SOLVENTS

Many methods exist for the purification of reagents and solvents. A number of these methods are routinely used in synthetic as well as analytical chemistry and biochemistry. These techniques, outlined below, will be discussed in greater detail in the respective sections in this Chapter. It is important to note that more than one method of purification may need to be implemented in order to obtain compounds of highest purity.
Common methods of purification are:
(a) Solvent Extraction and Distribution
(b) Distillation
(c) Recrystallisation
(d) Sublimation
(e) Chromatography

For substances contaminated with water or solvents, drying with appropriate absorbents and desiccants may be sufficient.

SOLVENT EXTRACTION AND DISTRIBUTION

Extraction of a substance from suspension or solution into another solvent can sometimes be used as a purification process. Thus, organic substances can often be separated from inorganic impurities by shaking an aqueous solution or suspension with suitable immiscible solvents such as benzene, carbon tetrachloride, chloroform, diethyl ether, diisopropyl ether or petroleum ether. After several such extractions the combined organic phase is dried and the solvent is evaporated. Grease from the glass taps of conventional separating funnels is invariably soluble in the solvents used. Contamination with grease can be very troublesome particularly when the amounts of material to be extracted are very small. Instead, the glass taps should be lubricated with the extraction solvent; or better, the taps of the extraction funnels should be made of the more expensive material *Teflon*. Immiscible solvents suitable for extractions are given in Table 1. Addition of electrolytes (such as ammonium sulfate, calcium chloride or sodium chloride) to the aqueous phase helps to ensure that the organic layer separates cleanly and also decreases the extent of extraction into the latter. Emulsions can also be broken up by filtration (with suction) through Celite, or by adding a little octyl alcohol or some other paraffinic alcohol. The main factor in selecting a suitable immiscible solvent is to find one in which the material to be extracted is readily soluble, whereas the substance from which it is being extracted is not. The same considerations apply irrespective of whether it is the substance being purified, or one of its contaminants, that is taken into the new phase. (The second of these processes is described as washing.)

Common examples of washing with aqueous solutions include the following:

Removal of acids from water-immiscible solvents by washing with aqueous alkali, sodium carbonate or sodium bicarbonate.

Removal of phenols from similar solutions by washing with aqueous alkali.

Removal of organic bases by washing with dilute hydrochloric or sulfuric acids.

Removal of unsaturated hydrocarbons, of alcohols and of ethers from saturated hydrocarbons or alkyl halides by washing with cold concentrated sulfuric acid.

This process can also be applied to purification of the substance if it is an acid, a phenol or a base, by extracting into the appropriate aqueous solution to form the salt which, after washing with pure solvent, is again converted to

the free species and re-extracted. Paraffin hydrocarbons can be purified by extracting them with phenol (in which aromatic hydrocarbons are highly soluble) prior to fractional distillation.
For extraction of solid materials with a solvent, a *Soxhlet* extractor is commonly used. This technique is applied, for example, in the alcohol extraction of dyes to free them from insoluble contaminants such as sodium chloride or sodium sulfate.
Acids, bases and amphoteric substances can be purified by taking advantage of their ionisation constants.

Ionisation constants and pK.
When substances ionise their neutral species produce positive and negative species. The ionisation constants are those constant values (equilibrium constants) for the equilibria between the charged species and the neutral species, or species with a larger number of charges (e.g. between mono and dications). These ionisation constants are given as **pK** values where **pK = -log K** and **K** is the dissociation constant for the equilibrium between the species [Albert and Serjeant *The Determination of Ionisation Constants*, A Laboratory Manual, 3rd Edition, Chapman & Hall, New York, London, 1984, ISBN 0412242907].

The advantage of using pK values (instead of K values) is that theory (and practice) states that the pK values of ionisable substances are numerically equal to the pH of the solution at which the concentrations of ionised and neutral species are equal. For example acetic acid has a pK^{25} value of 4.76 at 25° in H_2O, then at pH 4.76 the aqueous solution contains equal amounts of acetic acid [AcOH] and acetate anion [AcO^-], i.e. [AcOH]/[AcO^-] of 50/50. At pH 5.76 (pK + 1) the solution contains [AcOH]/[AcO^-] of 10/90, at pH 6.76 (pK + 2) the solution contains [AcOH]/[AcO^-] of 1/99 etc; conversely at pH 3.76 (pK - 1) the solution contains [AcOH]/[AcO^-] of 90/10, and at pH 2.76 (pK - 2) the solution contains [AcOH]/[AcO^-] of 99/1.

One can readily appreciate the usefulness of pK value in purification procedures, e.g. as when purifying acetic acid. If acetic acid is placed in aqueous solution and the pH adjusted to 7.76 {[AcOH]/[AcO^-] with a ratio of 0.1/99.9}, and extracted with say diethyl ether, neutral impurities will be extracted into diethyl ether leaving almost all the acetic acid in the form of AcO^- in the aqueous solution. If then the pH of the solution is adjusted to 1.67 where the acid is almost all in the form AcOH, almost all of it will be extracted into diethyl ether.

Aniline will be used as a second example. It has a pK^{25} of 4.60 at 25° in H_2O. If it is placed in aqueous solution at pH 1.60 it will exist almost completely (99.9%) as the anilinium cation. This solution can then be extracted with solvents e.g. diethyl ether to remove neutral impurities. The pH of the solution is then adjusted to 7.60 whereby aniline will exist as the free base (99.9%) and can be extracted into diethyl ether in order to give purer aniline.

See Table 2 for the pH values of selected buffers.

A knowledge of the pK allows the adjustment of the pH without the need of large excesses of acids or base. In the case of inorganic compounds a knowledge of the pK is useful for adjusting the ionic species for making metal complexes which could be masked or extracted into organic solvents [Perrin and Dempsey *Buffers for pH and Metal ion Control*, Chapman & Hall, New York, London, 1974, ISBN 0412117002], or for obtaining specific anionic species in solution e.g. $H_2PO_4^-$, HPO_4^{2-} or PO_4^{3-}.

The **pK** values that have been entered in Chapters 4, 5 and 6 have been collected directly from the literature or from compilations of literature values for organic bases [Perrin *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London, 1965, Supplement 1972, ISBN 040870408X; Albert and Serjeant *The Determination of Ionisation Constants*, A Laboratory Manual, 3rd Edition, Chapman & Hall, London, New York, 1984, ISBN 0412242907]; organic acids [Kortum, Vogel and Andrussow, *Dissociation Constants of Organic Acids in Aqueous Solution,* Butterworth, London, 1961; Serjeant and Dempsey, *Dissociation Constants of Organic Acids in Aqueous Solution,* Pergamon Press, Oxford, New York, 1979, ISBN 0080223397; and inorganic acids and bases [Perrin, *Ionisation Constants of Inorganic Acids and Bases in Aqueous Solution*, Second Edition, Pergamon Press, Oxford, New York, 1982, ISBN 0080292143]. Where literature values were not available, values have been predicted and assigned $\mathbf{pK_{Est}}$ ~. Most predictions should be so close to true values as to make very small difference for the purposes intended in this book. The success of the predictions, i.e. how close to the true value, depends on the availability of pK values for closely related compounds because the effect of substituents or changes in structures are generally additive [Perrin, Dempsey and Serjeant, *pKa Prediction for Organic Acids and Bases*, Chapman & Hall, London, New York, 1981, ISBN 041222190X].

All the pK values in this book are pKa values, the acidic pK, i.e. dissociation of H^+ from an acid (AH) or from a conjugate base (BH^+). Occasionally pKb values are reported in the literature but these can be converted using the equation **pKa + pKb = 14**. For strong acids e.g. sulfuric acid, and strong bases, e.g. sodium hydroxide, the pK values lie beyond the 1 to 11 scale and have to be measured in strong acidic and basic media. In these cases appropriate scales e.g. the H_0 (for acids) and H_- (for bases) have been used [see Katritzky and Waring *J Chem Soc* 1540 *1962*]. These values will be less than 1 (and negative) for acids and >11 for bases. They are a rough guides to the strengths of acids and bases. Errors in the stated pK and pK_{Est} ~ values can be judged from the numerical values given. Thus pK values of 4.55, 4.5 and 4 mean that the respective errors are better than ± 0.05, ± 0.3 and ± 0.5. Values taken from the literature are written as **pK**, and all the values that were estimated because they were not found in the literature are written as **pK_{Est}**.

pK and Temperature.

The temperatures at which the literature measurements were made are given as superscripts, e.g. pK^{25}. Where no temperature is given, it is assumed that the measurements were carried out at room temperature, e.g. 15—25°. No temperature is given for estimated values (**pK_{Est}** ~) and these have been calculated from data at room temperature. The variation of pK with temperature is given by the equation:

$$- d(pK)/dT = (pK + 0.052\Delta S^o)/T$$

where T is in degrees Kelvin and ΔS^o is in Joules deg^{-1} mol^{-1}. The -d(pK)/dT in the range of temperatures between 5 to 70° is generally small (e.g. between ~0.0024 and ~0.04), and for chemical purification purposes is not a seriously deterring factor. It does however, vary with the compound under study because ΔS^o varies from compound to compound. The following are examples of the effect of temperature on pK values: for imidazole the pK values are 7.57 (0°), 7.33 (10°), 7.10 (20°), 6.99 (25°), 6.89 (30°), 6.58 (40°) and 6.49 (50°), and for 3,5-dinitrobenzoic acid they are 2.60 (10°), 2.73 (20°), 2.85 (30°), 2.96 (40°) and 3.07 (40°), and for *N*-acetyl-β-alanine they are 4.4788 (5°), 4.4652 (10°), 4.4564 (15°), 4.4488 (20°), 4.4452 (25°), 4.4444 (30°), 4.4434 (35°) and 4.4412 (40°).

pK and solvent.

All stated pK values in this book are for data in dilute aqueous solutions unless otherwise stated, although the dielectric constants, ionic strengths of the solutions and the method of measurement, e.g. potentiometric, spectrophotometric etc, are not given. Estimated values are also for dilute aqueous solutions whether or not the material is soluble enough in water. Generally the more dilute the solution the closer is the pK to the real thermodynamic value. The pK in mixed aqueous solvents can vary considerably with the relative concentrations and with the nature of the solvents. For example the pK^{25} values for *N*-benzylpenicillin are 2.76 and 4.84 in H_2O and H_2O/EtOH (20:80) respectively; the pK^{25} values for (-)-ephedrine are 9.58 and 8.84 in H_2O and $H_2O/MeOCH_2CH_2OH$ (20:80) respectively; and for cyclopentylamine the pK^{25} values are 10.65 and 4.05 in H_2O and H_2O/EtOH (50:50) respectively. pK values in acetic acid or aqueous acetic acid are generally lower than in H_2O.

The dielectric constant of the medium affects the equilibria where charges are generated in the dissociations e.g. $AH \rightleftharpoons A^- + H^+$ and therefore affects the pK values. However, its effect on dissociations where there are no changes in total charge such as $BH^+ \rightleftharpoons B + H^+$ is considerably less, with a slight decrease in pK with decreasing dielectric constant.

DISTILLATION

One of the most widely applicable and most commonly used methods of purification of liquids or low melting solids (especially of organic chemicals) is fractional distillation at atmospheric, or some lower, pressure. Almost without exception, this method can be assumed to be suitable for all organic liquids and most of the low-melting organic solids. For this reason it has been possible in Chapter 4 to omit many procedures for purification of organic chemicals when only a simple fractional distillation is involved - the suitability of such a procedure is implied from the boiling point.

The boiling point of a liquid varies with the 'atmospheric' pressure to which it is exposed. A liquid boils when its vapour pressure is the same as the external pressure on its surface, its normal boiling point being the temperature at which its vapour pressure is equal to that of a standard atmosphere (760mm Hg). Lowering the external pressure lowers the boiling point. For most substances, boiling point and vapour pressure are related by an equation of the form,

$$\log p = A + B/(t + 273),$$

where p is the pressure, t is in °C, and A and B are constants. Hence, if the boiling points at two different pressures are known the boiling point at another pressure can be calculated from a simple plot of log p *versus* $1/(t + 273)$. For organic molecules that are not strongly associated, this equation can be written in the form,

$$\log p = 8.586 - 5.703\ (T + 273)/(t + 273)$$

where T is the boiling point in °C at 760mm Hg. Tables 3A and 3B give computed boiling points over a range of pressures. Some examples illustrate its application. Ethyl acetoacetate, **b** 180° (with decomposition) at 760mm Hg has a predicted **b** of 79° at 16mm; the experimental value is 78°. Similarly 2,4-diaminotoluene, **b** 292° at 760mm, has a predicted **b** of 147° at 8mm; the experimental value is 148-150°. For self-associated molecules the predicted **b** are lower than the experimental values. Thus, glycerol, **b** 290° at 760mm, has a predicted **b** of 146° at 8mm: the experimental value is 182°.

Similarly an estimate of the boiling points of liquids at reduced pressure can be obtained using a nomogram (see Figure 1).

For pressures near 760mm, the change in boiling point is given approximately by,

$$\hat{I}t = a(760 - p)(t + 273)$$

where $a = 0.00012$ for most substances, but $a = 0.00010$ for water, alcohols, carboxylic acids and other associated liquids, and $a = 0.00014$ for very low-boiling substances such as nitrogen or ammonia [Crafts *Chem Ber* **20** 709 *1887*]. When all the impurities are non-volatile, simple distillation is adequate purification. The observed boiling point remains almost constant and approximately equal to that of the pure material. Usually, however, some of the impurities are appreciably volatile, so that the boiling point progressively rises during the distillation because of the progressive enrichment of the higher-boiling components in the distillation flask. In such cases, separation is effected by fractional distillation using an efficient column.

Techniques.

The distillation apparatus consists basically of a distillation flask, usually fitted with a vertical fractionating column (which may be empty or packed with suitable materials such as glass helices or stainless-steel wool) to which is attached a condenser leading to a receiving flask. The bulb of a thermometer projects into the vapour phase just below the region where the condenser joins the column. The distilling flask is heated so that its contents are steadily vaporised by boiling. The vapour passes up into the column where, initially, it condenses and runs back into the flask. The resulting heat transfer gradually warms the column so that there is a progressive movement of the vapour phase-liquid boundary up the column, with increasing enrichment of the more volatile component. Because of this fractionation, the vapour finally passing into the condenser (where it condenses and flows into the receiver) is commonly that of the lowest-boiling components in the system. The conditions apply until all of the low-boiling material has been distilled, whereupon distillation ceases until the column temperature is high enough to permit the next component to distil. This usually results in a temporary fall in the temperature indicated by the thermometer.

Distillation of liquid mixtures.

The principles involved in fractional distillation of liquid mixtures are complex but can be seen by considering a system which approximately obeys *Raoult's law*. (This law states that the vapour pressure of a solution at any given temperature is the sum of the vapour pressures of each component multiplied by its mole fraction in the solution.) If two substances, A and B, having vapour pressures of 600mm Hg and 360mm Hg, respectively, were mixed in a molar ratio of 2:1 (i.e. 0.666:0.333 mole ratio), the mixture would have (ideally) a vapour pressure of 520mm Hg (i.e. 600 x 0.666 + 360 x 0.333, or 399.6 + 119.88 mm Hg) and the vapour phase would contain 77% (399.6 x 100/520) of A and 23% (119.88 x 100/520) of B. If this phase was now condensed, the new liquid phase would, therefore, be richer in the volatile component A. Similarly, the vapour in equilibrium with this phase is still further enriched in A. Each such liquid-vapour equilibrium constitutes a "theoretical plate". The efficiency of a fractionating column is commonly expressed as the number of such plates to which it corresponds in operation. Alternatively, this information may be given in the form of the height equivalent to a theoretical plate, or HETP. The number of theoretical plates and equilibria between liquids and vapours are affected by the factors listed to achieve maximum separation by fractional distillation in the section below on techniques.

In most cases, systems deviate to a greater or lesser extent from Raoult's law, and vapour pressures may be greater or less than the values calculated. In extreme cases (e.g. azeotropes), vapour pressure-composition curves pass through maxima or minima, so that attempts at fractional distillation lead finally to the separation of a constant-boiling (azeotropic) mixture and one (but not both) of the pure species if either of the latter is present in excess.

Elevation of the boiling point by dissolved solids. Organic substances dissolved in organic solvents cause a rise in boiling point which is proportional to the concentration of the substance, and the extent of rise in temperature is characteristic of the solvent. The following equation applies for dilute solutions and non-associating substances:

$$\frac{M\,\Delta t}{c} = K$$

Where M is the molecular weight of the solute, Δt is the elevation of boiling point in °C, c is the concentration of solute in grams for 1000gm of solvent, and K is the *Ebullioscopic Constant* (molecular elevation of the boiling point) for the solvent. K is a fixed property (constant) for the particular solvent. This has been very useful for the determination of the molecular weights of organic substances in solution.

The efficiency of a distillation apparatus used for purification of liquids depends on the difference in boiling points of the pure material and its impurities. For example, if two components of an ideal mixture have vapour pressures in the ratio 2:1, it would be necessary to have a still with an efficiency of at least seven plates (giving an enrichment of $2^7 = 128$) if the concentration of the higher-boiling component in the distillate was to be reduced to less than 1% of its initial value. For a vapour pressure ratio of 5:1, three plates would achieve as much separation.
In a fractional distillation, it is usual to reject the initial and final fractions, which are likely to be richer in the lower-boiling and higher-boiling impurities respectively. The centre fraction can be further purified by repeated fractional distillation.

To achieve maximum separation by fractional distillation:

1. The column must be flooded initially to wet the packing. For this reason it is customary to operate a still at reflux for some time before beginning the distillation.

2. The reflux ratio should be high (i.e. the ratio of drops of liquid which return to the distilling flask and the drops which distil over), so that the distillation proceeds slowly and with minimum disturbance of the equilibria in the column.

3. The hold-up of the column should not exceed one-tenth of the volume of any one component to be separated.

4. Heat loss from the column should be prevented but, if the column is heated to offset this, its temperature must not exceed that of the distillate in the column.

5. Heat input to the still-pot should remain constant.

6. For distillation under reduced pressure there must be careful control of the pressure to avoid flooding or cessation of reflux.

Types of distillation

The distilling flask. To minimise superheating of the liquid (due to the absence of minute air bubbles or other suitable nuclei for forming bubbles of vapour), and to prevent bumping, one or more of the following precautions should be taken:

(a) The flask is heated uniformly over a large part of its surface, either by using an electrical heating mantle or, by partial immersion in a bath above the boiling point of the liquid to be distilled.

(b) Before heating begins, small pieces of unglazed fireclay or porcelain (porous pot, boiling chips), pumice, diatomaceous earth, or platinum wire are added to the flask. These act as sources of air bubbles.

(c) The flask may contain glass siphons or boiling tubes. The former are inverted J-shaped tubes, the end of the shorter arm being just above the surface of the liquid. The latter comprise long capillary tubes sealed above the lower end.

(d) A steady slow stream of inert gas (e.g. N_2, Ar or He) is passed through the liquid.

(e) The liquid in the flask is stirred mechanically. This is especially necessary when suspended insoluble material is present.

For simple distillations a Claisen flask is often used. This flask is, essentially, a round-bottomed flask to the neck of which is joined another neck carrying a side arm. This second neck is sometimes extended so as to form a

Vigreux column [a glass tube in which have been made a number of pairs of indentations which almost touch each other and which slope slightly downwards. The pairs of indentations are arranged to form a spiral of glass inside the tube].

For heating baths, see Table 4. For distillation apparatus on a micro or semi-micro scale see Aldrich and other glassware catalogues. Alternatively, some useful websites for suppliers of laboratory glassware are www.wheatonsci.com, www.sigmaaldrich.com and www.kimble-kontes.com.

Types of columns and packings. A slow distillation rate is necessary to ensure that equilibrium conditions operate and also that the vapour does not become superheated so that the temperature rises above the boiling point. Efficiency is improved if the column is heat insulated (either by vacuum jacketing or by lagging) and, if necessary, heated to just below the boiling point of the most volatile component. Efficiency of separation also improves with increase in the heat of vaporisation of the liquids concerned (because fractionation depends on heat equilibration at multiple liquid-gas boundaries). Water and alcohols are more easily purified by distillation for this reason.
Columns used in distillation vary in their shapes and types of packing. Packed columns are intended to give efficient separation by maintaining a large surface of contact between liquid and vapour. Efficiency of separation is further increased by operation under conditions approaching total reflux, i.e. under a high reflux ratio. However, great care must be taken to avoid flooding of the column during distillation. The minimum number of theoretical plates for satisfactory separation of two liquids differing in boiling point by $\hat{I}t$ is approximately $(273 + t)/3\hat{I}t$, where t is the average boiling point in °C.
The packing of a column greatly increases the surface of liquid films in contact with the vapour phase, thereby increasing the efficiency of the column, but reducing its capacity (the quantities of vapour and liquid able to flow in opposite directions in a column without causing flooding). Material for packing should be of uniform size, symmetrical shape, and have a unit diameter less than one eighth that of the column. (Rectification efficiency increases sharply as the size of the packing is reduced but so, also, does the hold-up in the column.) It should also be capable of uniform, reproducible packing.

The usual *packings* are:
(a) Rings. These may be hollow glass or porcelain (Raschig rings), of stainless steel gauze (Dixon rings), or hollow rings with a central partition (Lessing rings) which may be of porcelain, aluminium, copper or nickel.
(b) Helices. These may be of metal or glass (Fenske rings), the latter being used where resistance to chemical attack is important (e.g. in distilling acids, organic halides, some sulfur compounds, and phenols). Metal single-turn helices are available in aluminium, nickel or stainless steel. Glass helices are less efficient, because they cannot be tamped to ensure uniform packing.
(c) Balls or beads. These are usually made of glass.

Condensers. Some of the more commonly used condensers are:
Air condenser. A glass tube such as the inner part of a Liebig condenser (see below). Used for liquids with boiling points above 90°. Can be of any length.
Coil condenser. An open tube, into which is sealed a glass coil or spiral through which water circulates. The tube is sometimes also surrounded by an outer cooling jacket. A double coil condenser has two inner coils with circulating water.
Double surface condenser. A tube in which the vapour is condensed between an outer and inner water-cooled jacket after impinging on the latter. Very useful for liquids boiling below 40°.
Friedrichs condenser. A "cold-finger" type of condenser sealed into a glass jacket open at the bottom and near the top. The cold finger is formed into glass screw threads.
Liebig condenser. An inner glass tube surrounded by a glass jacket through which water is circulated.

Vacuum distillation. This expression is commonly used to denote a distillation under reduced pressure lower than that of the normal atmosphere. Because the boiling point of a substance depends on the pressure, it is often possible by sufficiently lowering the pressure to distil materials at a temperature low enough to avoid partial or complete decomposition, even if they are unstable when boiled at atmospheric pressure.

Sensitive or high-boiling liquids should invariably be distilled or fractionally distilled under reduced pressure. The apparatus is essentially as described for distillation except that ground joints connecting the different parts of the apparatus should be air tight by using grease, or better Teflon sleaves. For low, moderately high, and very high temperatures Apiezon L, M and T greases respectively, are very satisfactory. Alternatively, it is often preferable to avoid grease and to use thin Teflon sleeves in the joints. The distilling flask, must be supplied with a capillary

bleed (which allows a fine stream of air, nitrogen or argon into the flask), and the receiver should be of the fraction collector type. When distilling under vacuum it is very important to place a loose packing of glass wool above the liquid to buffer sudden boiling of the liquid. The flask should be not more than two-thirds full of liquid. The vacuum must have attained a steady state, i.e. the liquid has been completely degassed, before the heat source is applied, and the temperature of the heat source must be raised *very slowly* until boiling is achieved.
If the pump is a filter pump off a high-pressure water supply, its performance will be limited by the temperature of the water because the vapour pressure of water at 10°, 15°, 20° and 25° is 9.2, 12.8, 17.5 and 23.8 mm Hg respectively. The pressure can be measured with an ordinary manometer. For vacuums in the range 10^{-2} mm Hg to 10 mm Hg, rotary mechanical pumps (oil pumps) are used and the pressure can be measured with a Vacustat McLeod type gauge. If still higher vacuums are required, for example for high vacuum sublimations, a mercury diffusion pump is suitable. Such a pump can provide a vacuum up to 10^{-6} mm Hg. For better efficiencies, the pump can be backed up by a mechanical pump. In all cases, the mercury pump is connected to the distillation apparatus through several traps to remove mercury vapours. These traps may operate by chemical action, for example the use of sodium hydroxide pellets to react with acids, or by condensation, in which case empty tubes cooled in solid carbon dioxide-ethanol or liquid nitrogen (contained in wide-mouthed Dewar flasks) are used.
Special oil or mercury traps are available commercially and a liquid-nitrogen (**b** -209.9°C) trap is the most satisfactory one to use between these and the apparatus. It has an advantage over liquid air or oxygen in that it is non-explosive if it becomes contaminated with organic matter. Air should not be sucked through the apparatus before starting a distillation because this will cause liquid oxygen (**b** –183°C) to condense in the liquid nitrogen trap and this is potentially explosive (especially in mixtures with organic materials). Due to the potential lethal consequences of liquid oxygen/organic material mixtures, care must be exercised when handling liquid nitrogen. Hence, it is advisable to degas the system for a short period before the trap is immersed into the liquid nitrogen (which is kept in a Dewar flask).

Spinning-band distillation. Factors which limit the performance of distillation columns include the tendency to flood (which occurs when the returning liquid blocks the pathway taken by the vapour through the column) and the increased hold-up (which decreases the attainable efficiency) in the column that should, theoretically, be highly efficient. To overcome these difficulties, especially for distillation under high vacuum of heat sensitive or high-boiling highly viscous fluids, spinning band columns are commercially available. In such units, the distillation columns contain a rapidly rotating, motor-driven, spiral band, which may be of polymer-coated metal, stainless steel or platinum. The rapid rotation of the band in contact with the walls of the still gives intimate mixing of descending liquid and ascending vapour while the screw-like motion of the band drives the liquid towards the still-pot, helping to reduce hold-up. There is very little pressure drop in such a system, and very high throughputs are possible, with high efficiency. For example, a 765-mm long 10-mm diameter commercial spinning-band column is reported to have an efficiency of 28 plates and a pressure drop of 0.2 mm Hg for a throughput of 330mL/h. The columns may be either vacuum jacketed or heated externally. The stills can be operated down to 10^{-5} mm Hg. The principle, which was first used commercially in the Podbielniak Centrifugal Superfractionator, has also been embodied in descending-film molecular distillation apparatus.

Steam distillation. When two immmiscible liquids distil, the sum of their (independent) partial pressures is equal to the atmospheric pressure. Hence in steam distillation, the distillate has the composition

$$\frac{\text{Moles of substance}}{\text{Moles of water}} = \frac{P_{substance}}{P_{water}} = \frac{760 - P_{water}}{P_{water}}$$

where the P's are vapour pressures (in mm Hg) in the boiling mixture.

The customary technique consists of heating the substance and water in a flask (to boiling), usually with the passage of steam, followed by condensation and separation of the aqueous and non-aqueous phases in the distillate. Its advantages are those of selectivity (because only some water-insoluble substances, such as naphthalene, nitrobenzene, phenol and aniline are volatile in steam) and of ability to distil certain high-boiling substances well below their boiling point. It also facilitates the recovery of a non-steam-volatile solid at a relatively low temperature from a high-boiling solvent such as nitrobenzene. The efficiency of steam distillation is increased if superheated steam is used (because the vapour pressure of the organic component is increased relative to water). In this case the flask containing the material is heated (without water) in an oil bath and the steam passing through it is superheated by prior passage through a suitable heating device (such as a copper coil heated electrically or an oil bath).

Azeotropic distillation. In some cases two or more liquids form constant-boiling mixtures, or azeotropes. Azeotropic mixtures are most likely to be found with components which readily form hydrogen bonds or are otherwise highly associated, especially when the components are dissimilar, for example an alcohol and an aromatic hydrocarbon, but have similar boiling points.

Examples where the boiling point of the distillate is a minimum (less than either pure component) include:
Water with ethanol, *n*-propanol and isopropanol, *tert*-butanol, propionic acid, butyric acid, pyridine,
methanol with methyl iodide, methyl acetate, chloroform,
ethanol with ethyl iodide, ethyl acetate, chloroform, benzene, toluene, methyl ethyl ketone,
benzene with cyclohexane,
acetic acid with toluene.
Although less common, azeotropic mixtures are known which have higher boiling points than their components. These include water with most of the mineral acids (hydrofluoric, hydrochloric, hydrobromic, perchloric, nitric and sulfuric) and formic acid. Other examples are acetic acid-pyridine, acetone-chloroform, aniline-phenol, and chloroform-methyl acetate.

The following azeotropes are important commercially for drying ethanol:

ethanol 95.5% (by weight) - water 4.5%	**b** 78.1°
ethanol 32.4% - benzene 67.6%	**b** 68.2°
ethanol 18.5% - benzene 74.1% - water 7.4%	**b** 64.9°

Materials are sometimes added to form an azeotropic mixture with the substance to be purified. Because the azeotrope boils at a different temperature, this facilitates separation from substances distilling in the same range as the pure material. (Conversely, the impurity might form the azeotrope and be removed in this way). This method is often convenient, especially where the impurities are isomers or are otherwise closely related to the desired substance. Formation of low-boiling azeotropes also facilitates distillation.

One or more of the following methods can generally be used for separating the components of an azeotropic mixture:

1. By using a chemical method to remove most of one species prior to distillation. (For example, water can be removed by suitable drying agents; aromatic and unsaturated hydrocarbons can be removed by sulfonation).
2. By redistillation with an additional substance which can form a ternary azeotropic mixture (as in ethanol-water-benzene example given above).
3. By selective adsorption of one of the components. (For example, of water on to silica gel or molecular sieves, or of unsaturated hydrocarbons onto alumina).
4. By fractional crystallisation of the mixture, either by direct freezing or by dissolving in a suitable solvent.

Kügelrohr distillation. The apparatus (Büchi, see www.buchi.com) is made up of small glass bulbs (*ca* 4-5cm diameter) which are joined together *via* Quickfit joints at each pole of the bulbs. The liquid (or low melting solid) to be purified is placed in the first bulb of a series of bulbs joined end to end, and the system can be evacuated. The first bulb is heated in a furnace at a high temperature whereby most of the material distils into the second bulb (which is outside of the furnace). The second bulb is then moved into the furnace and the furnace temperature is reduced by *ca* 5° whereby the liquid in the second bulb distils into the third bulb (at this stage the first bulb is now out at the back of the furnace and the third and subsequent bulbs are outside the front of the furnace). The furnace temperature is lowered by a further *ca* 5° and the third bulb is moved into the furnace. The lower boiling material will distil into the fourth bulb. The process is continued until no more material distils into the subsequent bulb. The vacuum (if applied) and the furnace are removed, the bulbs are separated and the various fractions of distillates are collected from the individual bulbs. For volatile liquids, it may be necessary to cool the receiving bulb with solid CO_2 held in a suitable container (Kügelrohr distillation apparatus with an integrated cooling system is available). This procedure is used for preliminary purification and the distillates are then redistilled or recrystallised.

Isopiestic or isothermal distillation. This technique can be useful for the preparation of metal-free solutions of volatile acids and bases for use in trace metal studies. The procedure involves placing two beakers, one of distilled water and the other of a solution of the material to be purified, in a desiccator. The desiccator is sealed and left to stand at room temperature for several days. The volatile components distribute themselves between the two beakers whereas the non-volatile contaminants remain in the original beaker. This technique has afforded metal-free pure solutions of ammonia, hydrochloric acid and hydrogen fluoride.

RECRYSTALLISATION

Techniques

The most commonly used procedure for the purification of a solid material by recrystallisation from a solution involves the following steps:

(a) The impure material is dissolved in a suitable solvent, by shaking or vigorous stirring, at or near the boiling point, to form a near-saturated solution.
(b) The hot solution is filtered to remove any insoluble particles. To prevent crystallisation during this filtration, a heated filter funnel can be used or the solution can be diluted with more of the solvent.
(c) The solution is then allowed to cool so that the dissolved substance crystallises out.
(d) The crystals are separated from the mother liquor, either by centrifuging or by filtering, under suction, through a sintered glass, a Hirsch or a Büchner, funnel. Usually, centrifugation is preferred because of the greater ease and efficiency of separating crystals and mother liquor, and also because of the saving of time and effort, particularly when very small crystals are formed or when there is entrainment of solvent.
(e) The crystals are washed free from mother liquor with a little fresh cold solvent, then dried.

If the solution contains extraneous coloured material likely to contaminate the crystals, this can often be removed by adding some activated charcoal (decolorising carbon) to the hot, but not boiling, solution which is then shaken frequently for several minutes before being filtered. (The large active surface of the carbon makes it a good adsorbent for this purpose.) In general, the cooling and crystallisation steps should be rapid so as to give small crystals which occlude less of the mother liquor. This is usually satisfactory with inorganic material, so that commonly the filtrate is cooled in an ice-water bath while being vigorously stirred. In many cases, however, organic molecules crystallise much more slowly, so that the filtrate must be set aside to cool to room temperature or left in the refrigerator. It is often desirable to subject material that is very impure to preliminary purification, such as steam distillation, Soxhlet extraction, or sublimation, before recrystallising it. A greater degree of purity is also to be expected if the crystallisation process is repeated several times, especially if different solvents are used. The advantage of several crystallisations from different solvents lies in the fact that the material sought, and its impurities, are unlikely to have similar solubilities as solvents and temperatures are varied.
For the final separation of solid material, sintered-glass discs are preferable to filter paper. Sintered glass is unaffected by strongly acid solutions or by oxidising agents. Also, with filter paper, cellulose fibres are likely to become included in the sample. The sintered-glass discs or funnels can be readily cleaned by washing in freshly prepared *chromic acid cleaning mixture*. This mixture is made by adding 100mL of concentrated sulfuric acid slowly with stirring to a solution of 5g of sodium dichromate (CARE: cancer suspect) in 5mL of water. (The mixture warms to about 70°, see p 3).
For materials with very low melting points it is sometimes convenient to use dilute solutions in acetone, methanol, pentane, diethyl ether or $CHCl_3$-CCl_4. The solutions are cooled to -78° in a dry-ice/acetone bath, to give a slurry which is filtered off through a precooled Büchner funnel. Experimental details, as applied to the purification of nitromethane, are given by Parrett and Sun [*J Chem Educ* **54** 448 *1977*].
Where substances vary little in solubility with temperature, *isothermal crystallisation* may sometimes be employed. This usually takes the form of a partial evaporation of a saturated solution at room temperature by leaving it under reduced pressure in a desiccator.
However, in rare cases, crystallisation is not a satisfactory method of purification, especially if the impurity forms crystals that are isomorphous with the material being purified. In fact, the impurity content may even be greater in such recrystallised material. For this reason, it still remains necessary to test for impurities and to remove or adequately lower their concentrations by suitable chemical manipulation prior to recrystallisation.

Filtration. Filtration removes particulate impurities rapidly from liquids and is also used to collect insoluble or crystalline solids which separate or crystallise from solution. The usual technique is to pass the solution, cold or hot, through a fluted filter paper in a conical glass funnel.
If a solution is hot and needs to be filtered rapidly a Büchner funnel and flask are used and filtration is performed under a slight vacuum (water pump), the filter medium being a circular cellulose filter paper wet with solvent. If filtration is slow, even under high vacuum, a pile of about twenty filter papers, wet as before, are placed in the Büchner funnel and, as the flow of solution slows down, the upper layers of the filter paper are progressively removed. Alternatively, a filter aid, e.g. Celite, Florisil or Hyflo-supercel, is placed on top of a filter paper in the funnel. When the flow of the solution (under suction) slows down, the upper surface of the filter aid is scratched gently. Filter papers with various pore sizes are available covering a range of filtration rates. Hardened filter papers are slow filtering but they can withstand acidic and alkaline solutions without appreciable hydrolysis of the

cellulose (see Table 5). When using strong acids it is preferable to use glass micro fibre filters which are commercially available (see Table 5 and 6).

Freeing a solution from extremely small particles [e.g. for optical rotatory dispersion (ORD) or circular dichroism (CD) measurements] requires filters with very small pore size. Commercially available (Millipore, Gelman, Nucleopore) filters other than cellulose or glass include nylon, Teflon, and polyvinyl chloride, and the pore diameter may be as small as 0.01micron (see Table 6). Special containers are used to hold the filters, through which the solution is pressed by applying pressure, e.g. from a syringe. Some of these filters can be used to clear strong sulfuric acid solutions.
As an alternative to the Büchner funnel for collecting crystalline solids, a funnel with a sintered glass-plate under suction may be used. Sintered-glass funnels with various porosities are commercially available and can be easily cleaned with warm chromic or nitric acid (see above).

When the solid particles are too fine to be collected on a filter funnel because filtration is extremely slow, separation by **centrifugation** should be used. Bench type centrifuges are most convenient for this purpose. The solid is placed in the centrifuge tube, the tubes containing the solutions on opposite sides of the rotor should be balanced accurately (at least within 0.05 to 0.1g), and the solutions are spun at maximum speed for as long as it takes to settle the solid (usually *ca* 3-5 minutes). The solid is washed with cold solvent by centrifugation, and finally twice with a pure volatile solvent in which the solid is insoluble, also by centrifugation. After decanting the supernatant, the residue is dried in a vacuum, at elevated temperatures if necessary. In order to avoid "spitting" and contamination with dust while the solid in the centrifuge tube is dried, the mouth of the tube is covered with aluminium foil and held fast with a tight rubber band near the lip. The flat surface of the aluminium foil is then perforated in several places with a pin and the tube and contents are dried in a vacuum desiccator over a desiccant.

Choice of solvents. The best solvents for recrystallisation have the following properties:
(a) The material is much more soluble at higher temperatures than it is at room temperature or below.
(b) Well-formed (but not large) crystals are produced.
(c) Impurities are either very soluble or only sparingly soluble.
(d) The solvent must be readily removed from the purified material.
(e) There must be no reaction between the solvent and the substance being purified.
(f) The solvent must not be inconveniently volatile or too highly flammable. (These are reasons why diethyl ether and carbon disulfide are not commonly used in this way.)

The following generalisations provide a rough guide to the selection of a suitable solvent:
(a) Substances usually dissolve best in solvents to which they are most closely related in chemical and physical characteristics. Thus, hydroxylic compounds are likely to be most soluble in water, methanol, ethanol, acetic acid or acetone. Similarly, petroleum ether might be used with water-insoluble substances. However, if the resemblance is too close, solubilities may become excessive.
(b) Higher members of homologous series approximate more and more closely to their parent hydrocarbon.
(c) Polar substances are more soluble in polar, than in non-polar, solvents.

Although Chapters 4, 5 and 6 provide details of the solvents used for recrystallising a large portion of commercially available laboratory chemicals, they cannot hope to be exhaustive, nor need they necessarily be the best choice. In other cases where it is desirable to use this process, it is necessary to establish whether a given solvent is suitable. This is usually done by taking only a small amount of material in a small test-tube and adding enough solvent to cover it. If it dissolves readily in the cold or on gentle warming, the solvent is unsuitable. Conversely, if it remains insoluble when the solvent is heated to boiling (adding more solvent if necessary), the solvent is again unsuitable. If the material dissolves in the hot solvent but does not crystallise readily within several minutes of cooling in an ice-salt mixture, another solvent should be tried.

Petroleum ethers are commercially available fractions of refined petroleum and are sold in fractions with about 20° boiling ranges. This ensures that little of the hydrocarbon ingredients boiling below the range is lost during standing or boiling when recrystallising a substance. Petroleum ethers with boiling ranges (at 760mm pressure) of 35—60°, 40—60°, 60—80°, 80—100°, and 100—120° are generally free from unsaturated and aromatic hydrocarbons. The lowest boiling petroleum ether commercially available has **b** 30-40°/760mm and is mostly *n*-pentane. The purer spectroscopic grades are almost completely free from olefinic and aromatic hydrocarbons. **Petroleum spirit** (which is sometimes used synonymously with petroleum ether or light

petroleum) is usually less refined petroleum, and *ligroin* is used for fractions boiling above 100°. The lower boiling fractions consist of mixtures of *n*-pentane (**b** 36°), *n*-hexane (**b** 68.5°) and *n*-heptane (**b** 98°), and some of their isomers in varying proportions. For purification of petroleum ether b 35-60° see p. 324.

Solvents commonly used for recrystallisation, and their boiling points, are given in Table 7.
For comments on the toxicity and use of **benzene** see the first pages of Chapters 4, 5 and 6.

Mixed Solvents. Where a substance is too soluble in one solvent and too insoluble in another, for either to be used for recrystallisation, it is often possible (provided they are miscible) to use them as a mixed solvent. (In general, however, it is preferable to use a single solvent if this is practicable.) Table 8 contains many of the common pairs of miscible solvents.
The technique of recrystallisation from mixed solvents is as follows:
The material is dissolved in the solvent in which it is the more soluble, then the other solvent (heated to near boiling) is added cautiously to the hot solution until a slight turbidity persists or crystallisation begins. This is cleared by adding several drops of the first solvent, and the solution is allowed to cool and crystallise in the usual way.
A variation of this procedure is simply to precipitate the material in a microcrystalline form from solution in one solvent at room temperature, by adding a little more of the second solvent, filtering off the crystals, adding a little more of the second solvent and repeating the process. This ensures, at least in the first or last precipitation, a material which contains as little as possible of the impurities, which may also be precipitated in this way. With salts, the first solvent is commonly water, and the second solvent is alcohol or acetone.

Recrystallisation from the melt. A crystalline solid melts when its temperature is raised sufficiently for the thermal agitation of its molecules or ions to overcome the restraints imposed by the crystal lattice. Usually, impurities weaken crystal structures, and hence lower the melting points of solids (or the freezing points of liquids). If an impure material is melted and cooled slowly (with the addition, if necessary, of a trace of solid material near the freezing point to avoid supercooling), the first crystals that form will usually contain less of the impurity, so that fractional solidification by partial freezing can be used as a purification process for solids with melting points lying in a convenient temperature range (or for more readily frozen liquids). Some examples of cooling baths that are useful in recrystallisation are summarised in Table 9. In some cases, impurities form higher melting eutectics with substances to be purified, so that the first material to solidify is less pure than the melt. For this reason, it is often desirable to discard the first crystals and also the final portions of the melt. Substances having similar boiling points often differ much more in melting points, so that fractional solidification can offer real advantages, especially where ultrapurity is sought. For further information on this method of recrystallisation, consult the earlier editions of this book as well as references by Schwab and Wichers (*J Res Nat Bur Stand* **25** 747 *1940*). This method works best if the material is already nearly pure, and hence tends to be a final purification step.

Zone refining. Zone refining (or zone melting) is a particular development for fractional solidification and is applicable to all crystalline substances that show differences in the concentrations of impurities in liquid and solid states at solidification. The apparatus used in this technique consists essentially of a device in which the crystalline solid to be purified is placed in a glass tube (set vertically) which is made to move slowly upwards while it passes through a fixed coil (one or two turns) of heated wire. A narrow zone of molten crystals is formed when the tube is close to the heated coil. As the zone moves away from the coil the liquid crystallises, and a fresh molten zone is formed below it at the coil position. The machine can be set to recycle repeatedly. At its advancing side, the zone has a melting interface with the impure material whereas on the upper surface of the zone there is a constantly growing face of higher-melting, resolidified material. This leads to a progressive increase in impurity in the liquid phase which, at the end of the run, is discarded from the bottom of the tube. Also, because of the progressive increase in impurity in the liquid phase, the resolidified material contains correspondingly more of the impurites. For this reason, it is usually necessary to make several zone-melting runs before a sample is satisfactorily purified. This is also why the method works most successfully if the material is already fairly pure. In all these operations the zone must travel slowly enough to enable impurities to diffuse or be convected away from the area where resolidification is occurring.
The technique finds commercial application in the production of metals of extremely high purity (impurities down to 10^{-9} ppm), in purifying refractory oxides, and in purifying organic compounds, using commercially available equipment. Criteria for indicating that definite purification is achieved include elevation of melting point, removal of colour, fluorescence or smell, and a lowering of electrical conductivity. Difficulties likely to be found with organic compounds, especially those of low melting points and low rates of crystallisation, are supercooling and, because of surface tension and contraction, the tendency of the molten zone to seep back into the recrystallised areas. The method is likely to be useful in cases where fractional distillation is not practicable, either because of

unfavourable vapour pressures or ease of decomposition, or where super-pure materials are required. It has been used for the latter purpose for purifying anthracene, benzoic acid, chrysene, morphine, 1,8-naphthyridine and pyrene to name a few. [See E.F.G.Herington, *Zone Melting of Organic Compounds*, Wiley & Sons, NY, 1963; W.Pfann, *Zone Melting*, 2nd edn, Wiley, NY, 1966; H.Schildknecht, *Zonenschmelzen*, Verlag Chemie, Weinheim, 1964; W.R.Wilcox, R.Friedenberg et al. *Chem Rev* **64** 187 *1964*; M.Zief and W.R.Wilcox (Eds), *Fractional Solidification*, Vol I, M Dekker Inc. NY, 1967.]

SUBLIMATION

Sublimation differs from ordinary distillation because the vapour condenses to a solid instead of a liquid. Usually, the pressure in the heated system is diminished by pumping, and the vapour is condensed (after travelling a relatively short distance) onto a cold finger or some other cooled surface. This technique, which is applicable to many organic solids, can also be used with inorganic solids such as aluminium chloride, ammonium chloride, arsenious oxide and iodine. In some cases, passage of a stream of inert gas over the heated substance secures adequate vaporisation. This procedure has the added advantage of removing occluded solvent used in recrystallising the solid.

CHROMATOGRAPHY

Chromatography is often used with advantage for the purification of small amounts of complex organic mixtures. Chromatography techniques all rely on the differential distribution of the various components in a mixture between the mobile phase and the stationary phase. The mobile phase can either be a gas or a liquid whereas the stationary phase can either be a solid or a liquid.

The major chromatographic techniques can also be categorised according to the nature of the mobile phase used - vapour phase chromatography for when a gas is the mobile phase and liquid chromatography for when a liquid is the mobile phase.

A very useful catalog for chromatographic products and information relating to chromatography (from gas chromatography to biochromatography) is that produced by Merck, called the ChromBook and the associated compact disk, ChromCircle.

Vapour phase chromatography (GC or gas-liquid chromatography)

The mobile phase in vapour phase chromatography is a gas (e.g. hydrogen, helium, nitrogen or argon) and the stationary phase is a non-volatile liquid impregnated onto a porous material. The mixture to be purified is injected into a heated inlet whereby it is vaporised and taken into the column by the carrier gas. It is separated into its components by partition between the liquid on the porous support and the gas. For this reason vapour-phase chromatography is sometimes referred to as gas-liquid chromatography (g.l.c). Vapour phase chromatography is very useful in the resolution of a mixture of volatile compounds. This type of chromatography uses either packed or capillary columns. Packed columns have internal diameters of 3-5 mm with lengths of 2-6 m. These columns can be packed with a range of materials including firebrick derived materials (chromasorb P, for separation of non polar hydrocarbons) or diatomaceous earth (chromasorb W, for separation of more polar molecules such as acids, amines). Capillary columns have stationary phase bonded to the walls of long capillary tubes. The diameters in capillary columns are less than 0.5 mm and the lengths of these columns can go up to 50 m! These columns have much superior separating powers than the packed columns. Elution times for equivalent resolutions with packed columns can be up to ten times shorter. It is believed that almost any mixture of compounds can be separated using one of the four stationary phases, OV-101, SE-30, OV-17 and Carbowax-20M. The use of capillary columns in gas chromatography for analysis is now routinely carried out. An extensive range of packed and capillary columns is available from chromatographic specialists such as Supelco, Alltech, Hewlett-Packard, Phenomenex etc.

Table 10 shows some typical liquids used for stationary phases in gas chromatography.

Although vapour gas chromatography is routinely used for the analysis of mixtures, this form of chromatography can also be used for separation/purification of substances. This is known as preparative GC. In preparative GC, suitable packed columns are used and as substances emerge from the column, they are collected by condensing the vapour of these separated substances in suitable traps. The carrier gas blows the vapour through these traps hence these traps have to be very efficient. Improved collection of the effluent vaporised fractions in preparative work is attained by strong cooling, increasing the surface of the traps by packing them with glass wool, and by applying an electrical potential which neutralises the charged vapour and causes it to condense.

When the gas chromatograph is attached to a mass spectrometer, a very powerful analytical tool (*gas chromatography-mass spectrometry*; **GC-MS**) is produced. Vapour gas chromatography allows the analyses of mixtures but does not allow the definitive identification of unknown substances whereas mass spectrometry is good for the identification of a single compound but is less than ideal for the identification of mixtures of

Next Page