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Water and wastewater analysis

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9.1 Introduction

This introduction provides an overview of water use and properties in textile manufacturing. The role of water in textiles, especially in wet processing, and sources and types of water, including raw material and wastewater, are briefly reviewed. Contaminants and impurities common to various types of water in textile manufacturing and the impact of contaminants are briefly described. In addition, water standards for various uses, for example, drinking water, raw water, process water, stream standards and wastewater are identified. Finally, general categories of water tests are listed and sampling, laboratory practices, limitations and potential improvements are noted.

The second part of this chapter presents descriptions of specific tests, including purpose and scope; accuracy, precision and correlation with key 'real-world' items and events; applicability and limitations; method employed; reporting and interpretation of results; and cost. Perspectives regarding anticipated future improvements in testing, with emphasis on issues pertaining to cost, accuracy and precision, correlation with 'real-world' items and events, applicability, analytical methods and sampling, and reporting and interpretation of results are also presented.

Finally, this chapter concludes with an annotated bibliography of references for the text as well as those that provide sources of further information and advice. Comments concerning the scope of various references are provided, to aid readers in their search for information regarding particular types of water or wastewater tests.

9.1.1 Water and textile wet processing – an overview

Textile wet processing is highly water intensive. Table 9.1 shows water consumption for processing of various types of textiles, based on a study of several hundred factories.

Water is used mainly in textile wet processing (preparation, coloration and

Table 9.1 Water quantities used in some types of textile wet processing (l kg^{-1})

Type of processing	Min	Av	Max
Wool finishing	4.2	11.7	77.6
Woven fabric preparation, coloration and finishing	5.0	113.4	507.9
Knit fabric preparation, coloration and finishing	20.0	83.4	377.8
Hosiery processing	5.8	69.2	289.4
Carpet processing	8.3	46.7	162.6

Source: USEPA, 1996.

finishing), and to a lesser extent in some other manufacturing operation steps in the textile production sequence, that is water jet weaving, synthetic fiber finishing, slashing, and the like. However, the vast majority of the water used is in the wet processing operations, and this is the focus of this chapter.

Water serves many purposes in textile processing, as indicated by Table 9.2.

In order to supply water to textile processes and other needs in textile manufacturing, there are many different water systems and sub-systems in a typical facility. These are listed in Table 9.3 below.

Perspectives regarding the importance of water

Water quality can have a significant effect on the efficiency and quality of textile processing. The effects may be positive or negative. Examples of interferences include the following (Smith, 1987):

- alkalinity or buffer systems: inhibits acid-catalyzed resin curing; increases hydrolysis rates for solutions of fiber reactive dyes;
- alum: flocking agent used in municipal water purification can be present as an impurity in water supplies, leading to filtering and spots on dyed goods, especially in beam and package dyeing;
- bacterial and organic chemical contamination: potable water supplies must be free of harmful contaminants;
- chlorine: many dyes, especially fiber reactive types, are severely degraded by the presence of residual chlorine used to disinfect drinking water, as is the common practice in municipal water systems;
- hardness: calcium and magnesium hardness ions cause precipitation of soaps, as well as saponification products (fatty acids) from scouring cellulosic fibers. Also, hardness causes precipitation of certain cellulosic dyes and causes difficulties in washing-off fiber reactive dyes;
- metals: iron, copper and other metal ions can cause undesirable decomposition of peroxide in bleach baths, resulting in foam, loss of bleaching effectiveness and pinholes in fabric. Heavy metals also can react with colorants, especially disperse dyes, to cause shade changes on dyed goods. In addition, less active

Table 9.2 Properties and roles of water in textile processing

Role	Comments or explanation
Solvent	Dissolves polar solutes to produce processing solutions
	Removes soluble impurities from substrates, washing
	Dissolves oxygen and other atmospheric gases (Henry's Law)
	Serves as media for homogeneous aqueous phase reactions
	Serves as liquid media for heterogeneous reactions and equilibria
	Serves as continuous phase for dispersions and emulsions
	Hydrates dyestuffs in solution, as well as dye sites in fibers
Chemical	Participates in hydrolysis and polymerization reactions
	Participates in buffer systems
	Promotes corrosion, especially if salt or acid is present
	Hosts galvanic reactions and ion exchange in metal parts
Electrical	Dielectric effects charge interactions in aqueous solutions
	Sorbed water increases conductivity and weight of substrates
	Humidity in processing areas reduces static electrical charges
	Immersed fibers can develop electrical charge (zeta potential)
Biological	Has no specific biological or nutrient activity per se
	Excellent medium for enzyme activity and biodegradation, e.g. of waste
	Develops osmotic pressure within cells, thus drives biological processes
	Dissolves nutrients
	Swells certain fibers
	Hosts biological growth
	Used for human consumption
Thermal	Efficient heat transfer fluid
	Cooling for machines, motors, bearings, etc
	Unusually large liquid range
	Relatively low viscosity, easy to pump, convective
	Conducts heat
	Steam (high enthalpy)
	Heat recovery from wastewater is common practice
Mechanical	Lubricant
	Transport substrates, e.g. in jet dyeing machines
Surface	Effective foaming medium, when used with surfactants
Polarity	Hydrogen bonding solvent
	Greater structure at low temperature, less structure at high temperature
	Hydrogen bonding structure can be chemically disrupted, e.g. with salt
Optical	High index of refraction, can make colors appear more intense
	Dissolves many dyestuffs (color)
	Can suspend particulates (turbidity)
Other	Moisture regain adds weight to textile substrates
	Fire suppression systems
	Environmental control in processing areas for comfort and efficiency

Table 9.3 Typical water systems/types in textile processing factories

Water type/system	Examples or comments
Process	Raw water from primary source
	Filtered/softened (cold) water, stored in clear wells
	Hot process water (preheated from heat recovery)
	Cold process wastewater
	Hot process wastewater (to heat recovery)
	Recycle/reuse process water (e.g. countercurrent wash water)
	Process bath reuse (e.g. dye/bleach bath, countercurrent washing)
	Chilled water
Waste treatment	Influent
	Sludge dewatering
	Effluent
Non-process	Non-contact cooling (recycle to clear well)
	Facility, equipment and implement wash-up
	Filter backwash
	Environmental (humidity) control in processing/storage areas
Laboratory	Distilled
	Deionized
	Aerated biological oxygen demand (BOD) dilution
Potable	For human consumption
Sanitary waste	From restrooms, food storage areas, etc.
Storm	Roof drains
	Parking lot runoff
Boiler	Feed water
	Steam
	Condensate return
	Blow-down
Recovered	From caustic or size recovery
	From wastewater treatment sludge dewatering
Closed systems	Cooling towers
Humidifier	Must be respirable quality

metal ions (e.g. copper) can undergo galvanic ion exchange reactions in metal plumbing systems to produce lead (from solder) and zinc (from galvanized pipe);

- organic chemicals: add color and/or odor to water, demand oxygen from solutions and can react with process chemistry, humans, enzymes, etc;
- reducing agents: reducing agents (e.g. oxygen demanding substances) such as dextrins can reduce and thereby decolorize dyes. This causes poor color repeats, e.g. in the pressure dyeing of polyester cotton blends with disperse and direct dyes;
- suspended solids: finely dispersed materials filter out in package and stock dyeing, producing sedimentary deposits on yarn and fiber;
- temperature: certain solutions used in textile processes are temperature

Table 9.4 Raw water quality in textile mills in the southeastern USA

Impurity	Average concentration for 10 mills studied
Ca^{2+}	12.9 mg l^{-1}
Mg^{2+}	3.8 mg l^{-1}
Na^{+}	36.0 mg l^{-1}
Alkalinity as $-CO_3^{2-}$	1.4 mg l^{-1}
Fe^{3+}	0.1 mg l^{-1}
Cu^{2+}	0.02 mg l^{-1}
Mn^{2+}	0.01 mg l^{-1}
Zn^{2+}	0.11 mg l^{-1}
pH	7.2 standard units

sensitive. For example, fiber reactive dyes hydrolyze more rapidly if made up hot, and mercerization solutions are more effective if the temperature is low.

The above are a few of the types of impact that water impurities can have on textile wet processes. There are many other effects, most of which are site-specific. Testing of water sources and processing solutions is a very important and often overlooked aspect of textile quality control. Typical data for contaminant levels in raw textile process water and in textile processing solutions are shown in Table 9.4.

9.1.2 Sources of water for textile operations

There are primarily four types of water sources for textile factories: surface water, groundwater, municipal systems and recovered/recycled water:

- Surface waters include such sources as rivers, lakes and shallow wells. These are typically contaminated with agricultural runoff, discharged pollutants from municipal and other waste treatment systems. In the USA, there are federal standards, as well as state standards that vary from state to state.
- Groundwater from deep wells typically contains particulates, suspended solids and dissolved solids, especially iron or hardness.
- Municipal or other public water systems typically have contaminants of two types: those that pass through the municipal purification system and those that are residues from the treatment process itself. Bacteria and turbidity are examples of the former. The latter may include materials such as alkalinity, alum, buffers, bromate, chlorine, chlorite, copper, haloacetic acids and trihalomethanes.
- Recycled and recovered water from reclaimed waste streams typically contain salt, surfactants, alkalinity, color and other contaminants that are common to the textile operation itself.

The impact of these contaminants on textile processes can be significant, as discussed above under ‘perspectives’. In the case of surface, ground or municipal

water, water quality is regulated in the USA by state, federal and sometimes local standards. Outside the USA, practices vary. An informative searchable database, maintained by the World Health Organization, contains detailed information on the water quality standards of many countries (http://www.who.int/water_sanitation_health/en).

9.1.3 Water standards

The quality of process water depends, of course, on its source. In the USA, sources are regulated as described below. (The state of North Carolina is used in the following as an example simply because it is a USA state with a significant textile industry. Owing to space limitations, it is not possible to include information from all 50 states.)

Drinking water standards (as produced by municipal systems) are set by the US Environmental Protection Agency (USEPA) and the individual states. National Primary Drinking Water Regulations apply to all public water systems and are designed to protect public health by reducing contaminants in drinking water (USEPA, 2000). National Secondary Drinking Water Regulations regulate contaminants that cause aesthetic effects (e.g. tooth discoloration, taste, odor, color). The EPA recommends these standards but does not enforce them. Some states have adopted them.

In the USA, groundwater standards are set at the state level. For example, the state of North Carolina regulates a list of 141 substances, as adopted by the North Carolina Environmental Management Commission. (North Carolina Administrative Code (NCAC) 2L.0202) In addition, nine other substances are listed in an interim standard (NCAC 2L.0202(c)) (North Carolina Administrative Code, 2002).

Stream standards are set by the states and, in some cases, may vary regionally within a state. Major contributors to contaminants in streams include agricultural and municipal storm water runoff and National Pollutant Discharge Elimination System (NPDES)-permitted point discharge sources. These are regulated by the USEPA under Title 40 CFR. For textile mills the relevant section is Part 410. This lists wastewater discharge limits for textile manufacturing sub-categories. North Carolina General Statutes (NCGS) for the protection of water resources are implemented by the NC Environmental Management Commission as well as the Department of Environment and Natural Resources (NCGS, 2000).

9.1.4 General types of water tests

There are many reasons for testing water, including compliance testing, process quality control, safety and health. There is one definitive source of water testing methods and that is *Standard Methods for the Examination of Water and Wastewater*, a joint publication of the American Public Health Association, The American Water Works Association, and the Water Pollution Control Federation

(Standard Methods, 1998). This reference contains methods of sampling as well as essentially every necessary water test in textile manufacturing. The most important types of tests in textile manufacturing include the following:

- Acidity, alkalinity, pH
- Biological content and contaminants
- Color and appearance
- Human and aquatic toxicity
- Metal ions
- Nutrients
- Organic materials, e.g. surfactants
- Oxygen demand
- Priority pollutants
- Solids.

Although seldom used in the textile area, the taste and odor of water can be assessed using standard methods. Specifically, these properties can be roughly quantified by the threshold odor method (2150 B) and the threshold taste method (2160 B) in which a water sample is diluted with pure odor- and taste-free dilution water until the odor or taste is just barely perceptible. This method is only roughly quantitative, owing to variances between observers, and time-to-time variations of the same observer. Samples collected for odor and taste testing should be collected and stored in completely full glass bottles with glass or tetrafluoroethylene (TFE)-lined closures. Storage should be under refrigeration. Odor tests at 60 ºC give increased sensitivity.

9.2 Samples and sampling

In addition to providing detailed procedures for sample collection for specific test methods, the 'Standard Methods' literature provides an overview of the general considerations associated with the collection and preservation of samples (Standard Method 1060). The principal goal is to ensure the collection of sufficient material for the test and representative of the material sampled yet small enough to be readily transported. A key purpose of sampling is often to determine compliance with regulatory standards. Methods developed for sampling include manual, automated and solid sorbent techniques.

9.2.1 General requirements

Samples are to be handled in a manner that prevents them from decomposing or being contaminated prior to their analysis. Consideration must be given to the filling of sample containers, collection and storage of composite samples, collection of samples containing metals, labeling of samples, description of sampling procedure, frequency of sampling, and the number and distribution of

sampling sites. Normally, sampling is to be avoided in areas of excessive turbulence, at weirs and at composite sites when volatile organic compounds (VOC) analysis is to be conducted. A summary of special sampling and handling requirements is provided in Table 1060:I of Standard Methods (1998).

Safety considerations

Adequate precautions must be taken when collecting and handling. This includes the use of protective apparel, gloves, safety glasses and well-ventilated areas. In the laboratory, sample containers are to be opened in a fume hood.

Types of samples

Test methods include specifications for the collection of grab samples, wastewater sludges, sludge banks, muds and composites. Advantages and disadvantages to composite sampling are reported in Standard Method 1060 B (Standard Methods, 1998). For instance, composite sampling is to be avoided when the components could undergo unavoidable changes during storage. Also, described in this method is the use of discharge-weighted methods. This type of sampling is associated with waters that vary in composition across their width or depth.

9.2.2 Laboratory practices, limitations and potential improvements

It is important to recognize that the various standardized methods developed for the analysis of water-based samples are not fail-proof. In this regard, the utility of specific methods and the reliability of test results are dependent upon the use of proper laboratory practices and understanding the limitations of a given method. This section is designed to illustrate some of the important issues in this area.

Chain of custody procedures

This aspect of the sampling process is critical to insuring the integrity of samples, from the collection stage to the reporting of test results. Consideration must be given to sample labels and seals, field and laboratory log books, chain of custody records, sample analysis requests, sample delivery to laboratory, scheduling of samples for analysis and disposal of samples following analyses.

Sample storage and preservation

While it is clear that is not always possible fully to maintain the original properties of a test sample, guidelines are provided in section 1060C of Standard Methods (1998) to help minimize errors introduced by improper storage and preservation of

samples. The key is to minimize the potential for volatilization or biodegradation between sampling and analysis.

Improper storage of samples can lead to changes in cation concentrations caused by adsorption on the surface of glass containers, leaching out of alkali from glass (thus changing the pH or alkalinity of samples), temperature-induced pH and dissolved gases changes, and changes in the chemical makeup of sample constituents caused by biological activity within the sample. Where possible, test samples should be analyzed on the day of collection. When this is not possible samples should be stored at 4 ºC.

Test method limitations

When employing a given test method, consideration must be given to the nature of the sample to be analyzed, as the presence of certain species either interferes with the detection of the target constituent or the concentrations of the target constituent may render a given method inappropriate. For instance, the presence of suspended matter and other ions that form precipitates with Ba^{2+} will give interferences leading to high results when making gravimetric determinations and colorimetric methods are preferred when non-turbid and colorless samples are employed.

9.3 Specific tests

Due to space limitations, all water tests that might be useful in textiles cannot be described here. The following selected tests are the ones that are most often used in textile manufacturing. These descriptions are abbreviated, and references are given in each case to more detailed explanations of the tests.

9.3.1 Physical and aggregate properties

There are a few very basic and general water tests that indicate important properties of water, but which do not detect the specific chemicals that are present. These include the following.

Alkalinity

The amount of alkalinity in water is typically determined by Standard Method 2320 (Standard Methods, 1998) and is reported as the equivalent amount of $CaCO_3$ in milligrams per liter. The purpose of alkalinity testing is to assess the presence of alkaline materials or buffer systems that might interfere with desired chemical reactions, or might promote underside reactions, as discussed in the introductory section of this chapter.

Samples are not filtered or altered in any way prior to alkalinity titration and all titrations are done at room temperature. Alkalinity may be measured as the amount

of standardized sulfuric or hydrochloric acid titer required to bring the sample to a specific pH end point. The pH may be detected by a well-calibrated pH meter, or by indicator materials. The most common indicators used are phenolphthalein (pH 8.3), *meta*-cresol purple (pH 8.3) or bromcresol green (pH 4.5). If the selected end point is pH 4.5, the alkalinity is reported as 'total alkalinity'. If the end point pH is 8.3, the alkalinity is reported as 'phenolphthalein alkalinity' regardless of the method used to detect the pH 8.3 end point. In the case of samples of very low alkalinity (less than 20 mg l^{-1}), the amount of standard acid required to decrease the pH by 0.3 units may be measured. This corresponds to a doubling of the hydrogen ion concentration. The amount of acid required to bring the pH to the desired end point then can be extrapolated from the result.

In some cases, color or turbidity of the sample may interfere with the visual observation of the end point color change if an indicator chemical is used. On the other hand, if a pH meter is used, certain surfactants, oils and the like can coat the pH probe and cause erroneous readings.

pH

pH, which is the negative logarithm of the hydrogen ion activity in a solution, is determined by a potentiometric method, using a well-calibrated commercial pH meter, as described in Standard Method 4500-H (Standard Methods, 1998). The purpose of pH testing is to detect the presence of the hydroxide or hydrogen ions that are required for various textile process, for example, fixation of fiber reactive dyes or acid dyeing of wool.

pH measurements must be done at room temperature, or using a temperature compensating instrument. Samples are not filtered nor altered in any way prior to measuring. The result is reported as a number, usually to one decimal place. Typically in textile processing solutions the pH value is between 4 and 10. Samples with pH values outside this range can be measured and reported but, owing to the logarithmic nature of the pH measurement unit, total alkalinity is often a better choice. In fact, even within the most appropriate working range for pH, alkalinity can reveal significant information which pH does not, for example, the presence of buffers. In some cases, certain surfactants, oils and the like can coat the pH probe and cause erroneous readings.

Temperature

Temperature measurements are used to characterize wastewater, as well as to make corrections for other tests that are temperature sensitive. Temperature is measured by allowing a thermometer or electronic device to come to thermal equilibrium with the water, by Standard Method 2550 (Standard Methods, 1998). Thermometers should be mercury in glass types, marked to a precision of 0.1 ºC. These should be well calibrated against primary standard thermometers, or against the

normal freezing point and boiling point of pure water. Thermometers are marked to indicate the proper depth for immersion and care must be taken to allow adequate time for the thermometer to equilibrate with the sample being measured.

Electronic methods include the use of well-calibrated commercial thermocouple- or thermistor -based instruments. These are typically built into meters used for other types of electrometric measurements, for example, pH and dissolved oxygen.

Conductivity

Conductance is the ability of water to transport electrical current, owing to the presence of dissolved ions, and is a very sensitive measure of high water purity. Conductance is traditionally measured as the reciprocal of resistance, in micromho's (mho), which is the reciprocal of megohms of resistance. Owing to variations in cell geometry, conductance measurements on a specific water sample vary and are reported as corrected to a standard geometry of 1 cm^2 of electrode surfaces placed parallel to each other and 1 cm apart. Since the conductance is generally proportional to the area of the electrodes and inversely related to the distance between them, the specific conductance is generally reported as mho cm^{-1}. The SI unit of conductance is the siemens, which is defined as one reciprocal ohm, and the usual reporting value for conductance is millisiemens per meter (mS m^{-1}). That being the case, one 10 mho cm^{-1} = 1 mS m^{-1}.

These measurements are made by Standard Method 2510 (Standard Methods, 1998) using a well-calibrated commercial instrument. Prior to measurement, the samples are brought to 25.0 ºC. Any contamination of the electrodes by oils, surfactants and the like can result in inaccurate readings.

Silica

Silica suspended in water can interfere with certain dyeing operations and can cause visible turbidity, as discussed in the introduction to this chapter. Various forms of silica can be determined by any one of several methods as described in Standard Method 4500-Si. For textile use, total silica is the most important. This may be determined gravimetrically by Standard Method 4500-Si-C or by atomic absorption spectrometry by Standard Method 3111D (Standard Methods, 1998). For information of atomic absorption methods, see the 'Metals' section below. Also, since the detrimental effects in textiles are related primarily to filtering of the silica on beam and package machines, it often suffices to measure the total suspended solids (TSS) in the water supply. TSS testing is described later in this chapter and is simpler than silica testing.

Total silica can be determined gravimetrically by Standard Method 4500-Si-C. In this method, hydrochloric acid decomposes the silica into forms that are insoluble and which precipitate. These precipitates are removed by filtration, then

dried at 110 °C and incinerated at 1200 °C. The residues are weighed, then treated with hydrofluoric acid solution, forming SiF_4. These are evaporated at 105 °C, with the addition of a small amount of perchloric acid to ensure complete dehydration. Upon further heating at 1200 °C, the SiF_4 evaporates and is determined by the weight loss. If other materials are known to be not present in the original sample, the weight of the residues upon the first drying and incineration can be taken as the amount of silica, as the entire amount of precipitated material will volatilize.

The method calls for the use of perchloric acid as a dehydrating agent. This is an explosive material and must be handled only by properly trained personnel. Care must be taken also to avoid contact of hydrofluoric acid and other solutions with glass, as glass is composed of silica and contact may contaminate the analysis.

Total hardness

Water hardness may be determined by Standard Method 2340 (Standard Methods, 1998). The results of the test are reported as the equivalent amount of $CaCO_3$ in the sample. There are two methods for this.

The first method is by calculation from independently determined Ca^{2+} and Mg^{2+} concentrations as described in the 'Metals' section below. The total hardness is calculated as follows:

$$\text{Total hardness, as } CaCO_3 \text{ equivalent mg } l^{-1} = 2.497\,[\text{Ca, mg } l^{-1}] + 4.118\,[\text{Mg, mg } l^{-1})]$$

The constants in the equation convert the concentration of Ca^{2+} and Mg^{2+} to the equivalent concentration of $CaCO_3$, where the atomic masses of Ca and Mg are 40.08 and 24.305, respectively, and the molecular mass of $CaCO_3$ is 100.09. Therefore, the factors needed to convert the Ca^{2+} and Mg^{2+} concentrations to the equivalent amount of $CaCO_3$ are 100.09/24.305 = 4.118 for Mg^{2+}, and 100.09/40.08 = 2.497 for Ca^{2+}.

The second method is by ethylene diamine tetra acetic acid (EDTA) titration, in which an indicator, Eriochrome Black T is added to the water sample, which develops a red color due to the presence of Ca^{2+}. When EDTA titer is added, Ca^{2+} is complexed and yields an end point at which the color changes to blue. To ensure a sharp end point, a small amount of magnesium salt of EDTA is added. The titration is performed at room temperature and at a pH of 10.

Some divalent metal ions can interfere including barium, cadmium, lead, manganese, strontium and zinc. These are titrated as hardness. In addition aluminum, cobalt, iron and nickel can interfere with the end point. This interference becomes more severe when phosphates are present above 10 mg l^{-1}. If these metals are present at significant levels, non-EDTA methods for hardness are preferred.

Oxidizing and reducing materials

The presence of oxygen in water is crucial for aquatic life. If discharged to the environment, wastewater that contains oxygen-demanding substances can deplete the oxygen in receiving waters, thereby damaging aquatic life. The measurement of oxygen content and oxygen demand of wastewater is critical to the operation of wastewater treatment systems and process chemical selection. The methods employed for determination of dissolved oxygen and determination of reducing materials are summarized in the sections that follow (i.e. dissolved oxygen biological oxygen demand and chemical oxygen demand).

Dissolved oxygen

Standard Method 4500-O describes two methods for determination of dissolved oxygen (DO) in water: Winkler's iodometric method and the elecrometric method (Standard Methods, 1998). The iodometric method is very accurate and precise, but the electrometric method is far more convenient for field use (e.g. in wastewater treatment system monitoring and control) and produces an electronic output that can easily be converted to digital form for microprocessor monitoring or control of wastewater treatment systems. Also, electrometric methods are not subject to certain interferences (i.e. oxidation or reduction of the iodine indicator). In addition, the iodometric method end point may be obscured by the presence of turbidity or color in textile wastewater samples. The electrochemical method is almost exclusively used in testing of textile wastewater.

The electrometric method (Standard Method 4500-O G) uses either an electrochemical- or a galvanic cell that interacts with the test sample through a membrane that allows dissolved oxygen to pass through. These cells and test instruments are commercially available from a wide variety of vendors. These use varying methods for calibration and temperature compensation, which must be followed explicitly to obtain accurate results.

The condition of the membrane is critical to the test. Physical damage, fouling by oils and surfactants, and other hazards require that the cell be renovated frequently by changing the membrane and the solution inside of the cell on a regular basis. In this regard, it is critical to follow the manufacturer's recommendation explicitly.

The electrometric DO test method can give measurements accurate to within 0.1 mg l^{-1}. These tests are very temperature sensitive, because temperature changes have a large effect on the diffusion of oxygen through the membrane. For this reason most commercial DO meters have built-in temperature compensation features.

High levels of salt can interfere with DO testing. The effects can be large and are also related to the temperature. Typical textile wastewaters from cotton dyeing have salt contents from 100 to 3000 mg l^{-1}. The upper end of this range can affect DO measurement.

Samples for DO testing must be collected and handled very carefully to ensure they are not exposed or shaken in air, which would alter their DO content. Also, samples taken from the surface of water may have a different DO content from samples taken from a greater depth. For this reason, most field instruments are equipped with cells designed to be immersed in the stream itself, rather than taking a sample. Also, it is best practice to analyze samples as soon as practical for DO, rather than storing them for long times prior to analysis. Since the electrometric method can be used to determine DO *in situ*, it can eliminate problems of sampling, handing and storage of samples for DO testing.

Biological oxygen demand

One of the most important characteristics of wastewater is the amount of oxygen required to stabilize it. This quantity is called the oxygen demand, and is determined either as biological oxygen demand (BOD) or chemical oxygen demand (COD). BOD is the quantity of oxygen required to stabilize wastewater in the presence of bacteria that consume the chemical pollutants and oxygen in the sample and can be determined by Standard Method 5210 (Standard Methods, 1998).

The test is conducted by placing the water sample (containing pollutants), plus oxygen buffer and nutrient solution (dissolved in the dilution water), and bacteria in an airtight container for a period of time. For research purposes, the time may vary, however, for NPDES or publicly-owned treatment works (POTW) compliance testing, the time is almost always five days. The sample is stored in an incubator to ensure that the temperature is constant at 20 ºC during the test. The dissolved oxygen content of the sample is determined initially and again after five days storage in an incubator with bacteria and nutrients. After applying certain correction factors, the depletion is converted into a biological oxygen demand (BOD) number, which is reported in milligrams of oxygen consumed per liter of undiluted sample.

The dilution water must contain the oxygen, nutrients and buffers for the test. This is prepared by adding pH 7.2 phosphate buffer solution and several nutrients (magnesium sulfate, calcium chloride and ferric chloride) to the water. This is then brought to 20 ºC in an incubator and thoroughly mixed with air by shaking or bubbling air into it. The DO content of dilution water should be about 8–9 mg l^{-1}. If the dilution water is of acceptable purity, it can be stored in an airtight container for five days with no more that 0.2 mg l^{-1} of oxygen depletion. Higher depletion indicates the presence of impurities in the water that oxidize and compromise the quality of the BOD test.

Owing to the variability of many factors in the BOD test (e.g. quality of the bacterial, see dilution water, incubator temperature), each batch of tests must include a control. The control is water containing 150 mg l^{-1} glucose plus 150 mg l^{-1} glutamic acid (GGA) and 2% of this GGA standard solution is introduced as a known

Table 9.5 Contents of typical test samples

	Contents	Comments
Dilution water blank	Dilution water only	DO depletion must be <0.2 mg l^{-1}
Seed blank	Seed plus dilution water	s = fraction of seed in the bottle
GGA control	Dilution water Seed 2% of GGA solution	t = fraction of seed in the test
Samples to be tested	Dilution water Seed Various amounts of sample	P = fraction of sample in the test

sample and must give a result of 198 ± 30.5 mg l^{-1}. If not, all results in the test batch are invalid.

The bacteria (called 'seed') for the test are generally available from the textile mill (or municipal POTW) waste treatment system. These bacteria, having been exposed to the textile factory wastewater for some time, are 'acclimated' to the pollutions that are present and therefore can degrade them and consume oxygen, which is the purpose of the test. The BOD of the seed itself must be determined by including 'seed blank' samples with each batch of tests.

Each test batch contains the following: water samples to be tested (diluted to various concentrations in test bottles), dilution water blanks, seed blanks and GGA control. Table 9.5 indicates the contents of typical samples. These samples are incubated together for five days. In some cases, longer term 20-day BOD tests may be performed. The DO in each bottle is measured before and after incubation. The BOD of the sample is determined using the following equation:

$$\text{BOD mg } l^{-1} = [\ (DO_{\text{before}} - DO_{\text{after}}) - (B_{\text{before}} - B_{\text{after}}) \times (t/s)\ ]/P$$

In this equation, DO_{before} is the DO in the sample bottle prior to incubation, DO_{after} is the DO in the sample bottle after incubation, B_{before} is the DO in the seed blank bottle prior to incubation, B_{after} is the DO in the seed blank after incubation, t is the fraction of seed in the test, s is the fraction of seed in the seed blank and P is the fraction of water sample in the test.

Samples must be analyzed as soon as practical after collection. If the sample must be stored, it must be refrigerated to avoid spoilage.

In all cases, DO depletion ($DO_{\text{before}} - DO_{\text{after}}$) of at least 2 mg l^{-1} must be observed for sample tests to be valid. If the DO depletion for all samples is less than 2 mg l^{-1}, it is possible that the sample contains materials that are toxic to the seed. Also, there must be a reasonable amount of DO remaining after incubation. If the DO_{after} is less than 2 mg l^{-1}, it is uncertain whether or not the sample would have consumed more DO, if present.

Owing to the inherent variability of many factors in this test, coefficient of variation of test results is about 10% within a laboratory. The differences between laboratories are expected to be greater owing to seed and dilution water variations.

Chemical oxygen demand

The chemical oxygen demand (COD) test may be performed by Standard Method 5220, which has three variants (Standard Methods, 1998). The most common method is 5220 D, the closed reflux colorimetric method. Like the BOD test described above, COD is a measure of the amount of oxygen required to stabilize wastewater. COD is the quantity of oxygen required to stabilize wastewater, reported as mg l^{-1} when exposed to a strong oxidizer, that is, dichromate.

COD testing has several advantages over BOD. Notably the COD test is more rapid, more repeatable, less susceptible to interferences and less labor intensive. On the other hand, BOD is more strongly correlated to processes that actually occur in wastewater treatment systems and receiving waters. For typical textile wastes, the COD:BOD ratio is typically about 3:1. Wastewater with higher ratios (e.g. 7:1) are resistant to aerobic biological treatment. (USEPA, 1996).

In the COD test, various amounts of water sample are added to a solution of dichromate and heated for some time, during which the pollutants in the water consume some of the dichromate. The residual dichromate is determined by titration or by visible spectrophotometry.

In the open reflux method (Standard Method 5220 B), 50 ml of sample (or some dilution of the sample) are put in a 250 ml ground glass stoppered Erlenmeyer flask along with 1 g of $HgSO_4$ and 5 ml of sulfuric acid reagent, composed of 5.5 g Ag_2SO_4 per kg H_2SO_4. The flask is fitted with a water cooled condenser, and 25 ml of 0.417 M potassium dichromate plus 70 ml additional sulfuric acid reagent is added. This mixture is refluxed for 2 h, then cooled. The residual mixture is titrated with nominally 0.25 M ferrous ammonium sulfate using a ferroin indicator (1,10 phenanthroline plus ferric sulfate). A blank, with no water sample in it, is also run. The COD is calculated by the following formula:

$$\text{COD mg } l^{-1} = (A - B)\, M\, 8000/(\text{ml sample in test})$$

In this equation, A is the ml of titer for the blank, B is the ml of titer for the sample and M is the exact molarity of the titrant.

The results are reported to the nearest mg l^{-1} and the coefficient of variation of test results is about 6–14%, depending on the level of COD present, based on interlaboratory studies (Standard Methods, 1998).

In the closed reflux spectrophotometric method (Standard Method 5220 D), a standardized colorimetric reagent, based on dichromate, is provided in a sealed vial. Test samples are added to the vial and heated to 150 ºC for 2 h in an aluminum block with appropriate holes to accommodate the vials. After cooling, the

transmission of the reaction mixture is measured at 600 m with a spectrophotometer. These instruments and supplies are available for several commercial vendors. The COD is determined from a calibration curve prepared from standardized potassium hydrogen phthalate solutions. The closed reflux methods retains volatiles and therefore can give more exact results. The coefficient of variation for this test is about 9%.

Total organic carbon

Total organic carbon (TOC) indicates the amount of carbon, regardless of its oxidation state prior to testing, whereas the COD or BOD test gives results that vary according to the oxidation state of the carbon (e.g. methane, methanol, formaldehyde, formic acid, carbon dioxide, sodium carbonate). This test provides a different type of information about the organic and inorganic carbon in a sample.

The method is typically performed by an instrument that oxidizes the sample to CO_2 over a catalyst, as described in Standard Method 5310 (Standard Methods, 1998). The amount of CO_2 produced is measured by an infrared analyzer. Calibrations are produced by injecting known materials into the analyzer. The accuracy for solutions is typically 1–2%. The accuracy for turbid samples with suspended matter is 5–10%.

Metals

There are many methods for metal determination (Standard Methods, 1998: section 3000). Some, for example as gravimetric, titrimetric or colorimetric methods, are most effective at high metal concentrations. Others, for example atomic absorption (AA), inductively coupled plasma (ICP) or inductively coupled plasma mass spectrometry (ICPMS) are far more sensitive. The latter are used for typical textile applications, such as compliance testing for water quality or detection of trace impurities in high-volume raw materials.

Metal ions of greatest interest in textiles are: antimony, arsenic, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, silver, sodium, tin, titanium and zinc.

ICP gives simultaneous determination of many metals, but the detection limits are typically not as low as AA. The actual detection limit varies depending on the specific metal. AA gives lower detection limits, but only analyzes one metal at a time, requiring equipment changes for additional metals. The most recently developed method is ICPMS, which simultaneously gives low detection limits with multiple metal detection capability (Cottingham, 2004). An EPA report (USEPA, 1998) compares five of the above test methods in detail. These were selected because of their low detection limits and because they use widely available, cost-effective technologies. The methods are:

- inductively coupled plasma/mass spectrometry (ICP/MS)

- stabilized temperature graphite furnace atomic absorption spectrophotometry (STGFAA)
- chelation preconcentration and ICP/MS
- chelation preconcentration and STGFAA
- hexavalent chromium by ion chromatography.

Owing to space limitations, all methods for metals cannot be presented here. Standard Methods presents the following methods for metals of interest in textiles. Numbers in parentheses are typical detection limits in g l^{-1} for AA and ICP. (Note: there are several AA methods and the detection limits presented are for the direct aspirational method. Other methods vary slightly.)

- colorimetric by Standard Method 3500: arsenic, chromium, copper, iron, lead, mercury, nickel, silver and zinc;
- titrimetric by Standard Method 3500: calcium and manganese;
- gravimetric by Standard Method 3500: magnesium;
- AA by Standard Method 3113: antimony (3), arsenic (1), chromium (2), cobalt (1), copper (1), iron (1), lead (1), manganese (2), nickel (1), silver (0.2) and tin (5);
- ICP (not ICPMS) by Standard Method 3120: antimony (30), arsenic (50), calcium (10), chromium (7), cobalt (7), copper (6), iron (7), lead (40), magnesium (30), manganese (2), nickel (15), silica (20 as SiO_2), silver (7), sodium (30) and zinc (2).

9.3.2 Color and appearance

The appearance of water, especially textile wastewater is systematically described in terms of its visible characteristics (Standard Methods, 1998). In this regard, the presence of color, suspended particles and turbidity is the focus of much of the testing conducted. In the case of textiles, the presence of color in wastewater is extremely important and numerical methods are normally employed to report results from making color assessments. While color in textile wastewater may arise from the presence of transition metal ions, vegetable matter and industrial plant effluents, color derived from unspent dyebaths is of primary importance. Invariably, this color is removed using a number of physical and/or chemical methods (Reife, 1993); however, methods enabling the recycle/reuse of dye-based color have been developed (Reife and Freeman, 1996).

Color assessments include visual and spectrophotometric methods, both of which employ reference standards to aid in the communication of results. For instance, the visual method involves a comparison of the color of the test sample with known concentrations of potassium chloropalatinate (K_2PtCl_5) and cobalt (II) chloride ($CoCl_2$) in distilled water. The estimated color of the test sample is then used to calculate color units, using the following equation:

$$\text{Color units} = \frac{A \times 50}{B}$$

where A is the estimated color and B is the volume of stock color standard employed.

In the visual comparison method, standards having colors of 5–70 are prepared by diluting 0.5–7.0 ml stock color standard with distilled water in Nessler tubes (Standard Method 2120 B). These solutions must be protected against evaporation and contamination.

In the spectrophotometric method, color is assessed by means of a standard absorption spectrophotometer. In this case, a description of the hue, intensity and brightness of a non-turbid colored solution is possible in the visible region (400–700 nm) of the electromagnetic spectrum. Since pH will have a significant impact on the color of certain dyes, the pH of the dye solution employed must be measured and reported. Details regarding the method are provided in section 2120 C of the Standard Methods book, and can be summarized as follows:

1 Make all measurements at room temperature.
2 Record spectra at the original pH of the sample and at pH 7.6 (adjusting the pH with H_2SO_4 or NaOH).
3 Filter to remove insoluble material.
4 Record the visible spectrum.

The American Dye Manufacturers Institute (ADMI) developed a method for measuring sample color independent of hue. This method provides a mechanism for assessing differences in samples that have color characteristics that are significantly different from the Pt–Co standards described above. This is Standard Method 2120E, which determines the percentage of light transmitted through a set of tristimulus light filters. The resultant transmission values are used to calculate tristimulus ($X_s Y_s Z_s$, intermediate (DE) and Munsell color values.

The American Public Health Association (APHA) also developed a method for evaluating the color of wastewater. Initially, this method was used as an indication of water purity and involved making comparisons of test samples with dilutions of a 500-ppm Pt–Co stock solution. In the APHA index system, distilled water is assigned a value of 0 (zero) and the stock Pt–Co solution has a value of 500. Details pertaining to the preparation of solutions and sample measurements are provided in ASTM D1209-93. In addition, ASTM D1209 describes how to correlate data from color measurement instruments with data from physical APHA and Pt–Co color standards.

9.3.3 Biological and microbiological methods

Biological methods for testing water quality emphasize the collection (sampling) and identification of aquatic organisms. Methods of this type aid in the determina-

tion of (1) the cause of color, odor, and taste in water samples; (2) the biological effects of pollution; (3) the progress of self-cleansing of bodies of water; (4) the effectiveness of wastewater treatment methods; and (5) the environmental impact of various natural and human activities. To make these assessments, the status of plankton, peri- and macro-phyton, microinvertebrates, fish and amphibian populations is often measured. The importance of plankton in the total aquatic ecosystem has led to the long-standing use of these microscopic, free-floating organisms as an indication of water quality (Standard Methods, 1998: section 10200). Their short life cycles cause them to respond quickly to environmental changes, making them a good indicator of the surrounding water quality. They are found in fresh and salt water and methods employed in sampling are also reported in Standard Methods (1998, section 10200).

Typical sample sizes are 0.5–1 l. However, water samples expected to have low densities of plankton should be collected in larger amounts (up to 6 l). Plankton nets are preferred over bottles and traps when sampling low density areas. For quick sample collections, a pump is used, giving preference to diaphragm and peristaltic pumps over centrifugal pumps because the latter can damage the organisms. The organisms contained in collected samples are concentrated prior to analyses, using sedimentation, membrane filtration or centrifugation techniques, with sedimentation being the preferred method.

Plankton are counted by using a counting cell or chamber which has a volume and area that facilitates the determination of population densities. The Sedgwick Rafter (S–R) cell is commonly employed, owing to ease of use and reproducibility. However, it is not suitable for nanoplankton because objectives providing high magnification cannot be used. In this case, the Palmer Maloney (P-M) nanoplankton cell was developed and is used despite having its own limitations.

The key purpose of developing methods for microbiological assessments of water is to determine its sanitary quality (Standard Methods, 1998: section 9010). In this regard, the methods developed provide for the detection and listing of indicator organisms, with the presence/absence of coliform bacteria serving as the primary indicator of the suitability of water for various end uses. Two principal methods have been developed for the determination of coliform bacteria levels: (1) the membrane filter method, which involves a direct plating technique for the detection and estimation of coliform densities and (2) the multiple-tube fermentation test, in which the results are reported as a most probable number (MPN) index. The MPN is the most probable number of coliform bacteria that would give the results obtained in the laboratory examination. Unlike direct plating methods such as the membrane filter procedure, it does provide a direct count of coliform colonies. Both methods provide estimates of the density of fecal organisms. This is important because fecal streptococci and enterococci are indicators of water sanitary quality and coliform bacteria is present in the feces of warm-blooded animals.

Microbiological methods have also been developed for the differentiation of the

coliform group, for examination of waters in swimming pools and for the isolation of certain pathogenic bacteria. Samples containing residual chlorine are typically treated with a reducing agent such as sodium thiosulfate ($Na_2S_2O_3$) prior to testing, to ensure an accurate indication of microbial content. Sample sizes of not less than 100 ml are employed.

In the membrane filtration method (Standard Method 9211 B), the test sample is passed through a membrane filter and the filter is placed on the surface of a plate containing M-7 FC agar medium. After incubating for 7 h at 41.5 ºC, the presence of yellow fecal coliform colonies (from lactose fermentation within the FC agar) serves as a positive test.

9.3.4 Organics (priority pollutants)

The control of toxic pollutants is essential to achieving the goals of the Clean Water Act (CWA) because elevated levels of toxic pollutants can accumulate in the tissue of aquatic organisms (especially fish and shellfish) and result in fishing/harvesting advisories or bans (USEPA, 2000). The CWA is designed to ensure all waters are sufficiently clean to protect public health and/or the environment. Consequently, established water quality goals enhance the effectiveness of state and federal water programs that have an impact on aquatic ecosystems and human health, including permitting, coastal water quality improvement, fish tissue quality protection, non-point source controls, drinking water quality and ecological protection.

Water quality standards provide numeric criteria for priority toxic pollutants for each state. For instance, the NPDES permits issued for San Francisco Bay were to be limited to levels not to exceed 5 ppb as a 4-day average and 20 ppb as a 1-h average for fresh water. The combination of a criterion maximum concentration (CMC), which is a short-term concentration limit, and a criterion continuous concentration (CCC), which is a 4-day average concentration limit, is designed to provide protection for aquatic life and its uses from acute and chronic toxicity. Consequently, the terms CMC and CCC refer to the acute and chronic toxicity criteria (values) for a given pollutant. The two-number criteria are designed to identify average pollutant concentrations that will produce water quality generally suited to maintenance of aquatic life and designated uses, while restricting the duration of occurrences exceeding the average. In this way, the total exposure to toxic pollutants will not cause unacceptable adverse effects.

The organic toxic pollutants monitored include: acrolein, acrylonitrile, carbon tetrachloride, chlorobenzene, 1,2-dichloroethane, 1,2-dichloroethylene, 1,3-dichloropropylene, ethylbenzene, 1,1,2,2-tetrachloroethane, tetrachloroethylene, 1,1,2-trichloroethane, trichloroethylene, vinyl chloride, 2,4-dichlorophenol, 2-methyl-4,6-dinitrophenol, 4,6-dinitrophenol, benzidine, bis-(2-chloroethyl)ether, bis(2-ethylhexyl)phthalate, 3,3'-dichlorobenzidine, diethylphthalate, dimethylphthalate, di-*n*-butylphthalate, 2,4-dinitrotoluene, 1,2-diphenylhydrazine,

hexachlorobutadiene, hexachlorocyclopentadiene, hexachloroethane, isophorone, nitrobenzene, *N*-nitrosodimethylamine, and *N*-nitrosodiphenylamine.

Samples containing chlorinated organics, phenols and aromatic amines are analyzed using liquid–liquid extractions followed by gas chromatography (GC)- or liquid chromatography (LC)-mass spectrometry (MS). In the case of chlorinated compounds, extractions are conducted using solvents such as hexanes, *t*-butyl ether and methylcyclohexane. This is followed by GC analysis, in which the retention times of the components are determined and compared with those of compounds in a reference library. When used in combination with mass spectrometry, molecular mass information is obtained and the resultant spectra are also compared against those in a reference library. The detection levels are 0.25–30 g l^{-1}, depending on the specific type of compound. For samples containing phenols or aromatic amines, extractions are performed using methylene chloride followed by GC- or LC-MS analysis.

When trouble shooting, liquid–liquid extractions with methylene chloride at acidic, neutral and alkaline pH are conducted, followed by neutralization, concentration and GC or GC/LC-MS analyses. This approach is highly effective for unsulfonated organic amines, phenols, carboxylic acids and neutral organics when extensive libraries of retention times and mass spectral data are available.

9.3.5 Solids

Solids

Proper wastewater treatment and drinking water purification system operation depends on solids measurements. There are several types of solids of interest presented in Standard Methods (1998):

- 2540B: Total solids
- 2540D: Total suspended solids
- 2540C: Total dissolved solids
- 2540F: Fixed and volatile solids
- 2540F: Settlability and buoyancy of solids.

Total solids

Standard Method 2540B determines the total solids (TS) in water samples by drying at 105 ºC to constant weight. The results are reported in mg l^{-1}, and typically the repeatability of the test is within 6 mg l^{-1}. In some cases where the solids are hygroscopic, the time required for drying may be long and the samples must be cooled before weighing in a dessicator to avoid sorption of moisture from the air.

Total dissolved solids

Standard Method 2540C determines total dissolved solids (TDS) by filtering a water sample through glass fiber disks, then drying the sample at 180 ºC for 1 h. The sample is cooled in a dessicator and weighed, then redried at 180 ºC. This process is repeated until a constant weight is obtained. Coefficient of variation of test values is 7%.

Total suspended solids

Standard Method 2540 D determines total suspended solids (TSS) by filtering a water sample through glass fiber disks, then drying the disc and filtered solids at 105 ºC to constant weight. The resulting test data have a coefficient of variation that is highly dependent on the level of solids in the original sample.

Fixed and volatile solids

For some applications, it is important to distinguish between volatile and non-volatile solids. For example, the bioactive organic portion of mixed liquor suspended solids in secondary activated sludge textile wastewater treatment systems is volatile, whereas grit and other inorganic solids are non-volatile. To determine the volatile component of solids from any of the above three tests, the solid residue is dried to constant weight in a high-temperature muffle furnace at 550 ºC (Standard Method 2540 F).

Settleable solids

Standard method 2540 F determines settleable solids either gravimetrically or volumetrically. In the volumetric method, a 1 l water sample is allowed to settle for 45 min in an Imhoff cone, then is very gently stirred to loosen any settleable particulates that adhere to the sides of the cone. After an additional 15 min of settling, the volume of the settled material is estimated from the marking on the cone. Do not count floating solids.

In the gravimetric method, the TSS is determined from the original sample and also from the supernatant liquid above the settled material. The settleable solids are computed as:

$$\text{settleable solids (mg l}^{-1}) = \text{initial TSS (mg l}^{-1}) - \text{supernatant TSS (mg l}^{-1})$$

9.3.6 Anions

Chloride (Standard Method 4500 Cl^-)

Several methods are available for Cl^- determination, with the choice of method

based largely on personal preference. The argentometric method is suitable for clear waters containing 0.15–10 mg Cl^- in the titrated sample. However, the end point of the mercuric nitrate method has been judged as easier to detect. In this case, special notice must be given to handling of mercury-based wastes. The potentiometric method is suitable for colored or turbid samples that would pose problems with visualization of color-based end points. The ferricyanide and flow-injection methods are automated, with the latter especially useful for large numbers of samples. Cl^- can also be determined using the capillary ion electrophoresis method (Standard Method 4140).

Nitrite (Standard Method 4500 NO_2^-)

Methods developed for NO_2^- determination include a colorimetric method and ion chromatography. In the colorimetric method, NO_2^- ion levels are determined through the formation of an azo red dye from a reagent containing sulfanilamide and *N*-(1-naphthyl)ethylenediamine at pH 2.0–2.5. The applicable range of the method for spectrophotometric measurements is 10–1000 g NO_2^- l^{-1}.

Single-column ion chromatography measurements afford a retention time of 3.1 min for NO_2^-. In this method the minimum detection limit (MDL) is 0.022 mg l^{-1}.

Nitrate (Standard Method 4500 NO_3^-)

Spectroscopic measurements for detecting NO_3^- are often complicated by the presence of interfering components. Its primary absorbance is at 220 nm, which overlies a number of organic compounds, making this method suitable when the concentration of organics is very low. UV absorption measurements have shown that an NO_3^- calibration curve follows Beer's law at levels up to 11 mg l^{-1}. This allows rapid determination of NO_3^- levels. Single-column ion chromatography measurements have been used and afford a retention time of 5.3 min for NO_3^-. In this method the minimum detection limit (MDL) is 0.035 mg l^{-1}. A nitrate electrode method has also been developed. This selective sensor method responds to NO_3^- ion activity in the 10^{-5}–10^{-1} M region, which corresponds to 0.14–1400 mg NO_3^- N l^{-1}.

Sulfide (Standard Method 4500 S^{2-})

Tests for S^{2-} include the antimony test, silver–silver sulfide electrode test and the lead acetate paper and silver foil tests. In the antimony test, the color produced by treating a 200 ml test sample with 0.5 ml saturated potassium antimony tartrate and 0.5 ml 6 N HCl is compared with colors produced when solutions containing known amounts of S^{2-} are treated in the same way. In the silver–silver sulfide electrode test, the test sample is diluted 1:1 with an alkaline solution of an oxidizing agent and the electrode potential relative to a double-junction reference electrode is measured.

S^{2-}levels are then estimated using a calibration curve. In the third method, the formation of PbS or Ag_2S serves as a positive test for the presence of S^{2-}.

Sulfite (Standard Method 4500 SO_3^{2-})

Iodometric titration is suitable for relatively clean waters having > 2 mg SO_3^{2-} l^{-1}. The phenanthroline colorimetric method is preferred at lower levels. In the former method, an acidic sample containing sulfite is titrated with a standardized solution of potassium iodide/iodate. The end point is signaled by the formation of a persistent blue color formed by the interaction of excess iodine with a starch indicator. In the phenanthroline method, SO_2 is produced and, in turn, converts ferric ions to ferrous ions which form an orange complex with phenanthroline. The complex is measured colorimetrically at 510 nm.

Sulfate (Standard Method 4500 SO_4^{2-})

Chromatographic, electrophoresis, gravimetric and turbidimetric methods are available for determining sulfate, the choice of which often varies with the sulfate levels present and the number of samples to be analyzed. The accuracy of gravimetric and turbidimetric methods has caused them to be preferred. In the gravimetric methods, sulfate is precipitated as $BaSO_4$ by the addition of $BaCl_2$ in HCl near the boil. The precipitate is collected by filtration, washed free of Cl^-, ignited or dried, and weighed to determine the amount of $BaSO_4$. In the turbidimetric method, sulfate is precipitated from an acetic acid solution to give $BaSO_4$ crystals of uniform size. The resultant suspension of $BaSO_4$ is measured using a photometer and the SO_4^{2-} level is determined by comparison of the reading with a standard curve.

Phosphorus (Standard Method 4500 P)

Phosphorus exists predominantly in water samples as phosphates, including orthophosphates, condensed phosphates and organophosphates. The analysis of samples for phosphorus levels involves conversion of the phosphorus species present to orthophosphate followed by colorimetric determination of the orthophosphate. In the case of organophosphorus compounds, a digestion method may be employed to oxidize the organic matter and generate orthophosphate. Oxidizing agents employed include HNO_3/H_2SO_4 and persulfate/UV light. Among the options for colorimetric analysis, the vanadomolybdophosphoric acid, stannous chloride and ascorbic acid methods are most widely used, the choice of which depends on the phosphorus levels present. The vanadomolybdophosphoric acid method is preferred at 1–20 mg P l^{-1} and the stannous chloride and ascorbic acid methods are preferred at 0.01–6 mg P l^{-1}. For further details, see Table 4500-P:I in Standard Methods (1998).

9.4 Laboratory practices

9.4.1 Sample storage and preservation

Metal ions based on aluminum, cadmium, chromium, copper, iron, lead, manganese, silver and zinc are subject to loss by adsorption on the walls of glass containers and should be (1) collected in separate clean bottles and (2) acidified with HNO_3 to pH <2 (to minimize surface adsorption and precipitation). Zero head space is important in preserving samples containing VOCs, making complete filling of containers important when this determination is to be conducted. The time interval between collection and analysis should be minimized. In the case of composite samples, field testing is preferred and the collection time is often specified as the time at the end point of the collection. When immediate testing is not practical, samples should be stored at 4 ºC. Significantly colder temperatures may cause a pH change in the samples and/or damage the sample containers, especially glass. The use of chemical preservatives must be restricted to cases in which the agent used does not interfere with the analysis to be made. Formaldehyde is to be avoided. Normally, the purpose of preservatives is to retard biological activity that, for instance, alters the oxidation state of sample constituents, or retards the hydrolysis of chemical constituents in the sample. A list of preservation methods is provided in Table 1060:I of Standard Methods (1998).

9.4.2 Method development

Method development comes into play when an established standard method does not exist for a particular sample constituent. In this case, a set of experimental steps for measuring a known amount of a given constituent in various matrices is developed. For the new method to become accepted by the scientific community, it must be validated. Validation consists of three steps: (1) determination of the MDL, (2) analysis of independently prepared standards and (3) determination of the stability of the result produced when steps comprising the new method are varied (method ruggedness). New methods passing these three tests are subjected to collaborative testing prior to becoming a standard method.

9.4.3 Expression of results

The units employed are normally based on the International System of Units (SI units) and physical results are expressed in milligrams per liter (mg l^{-1}) unless the constituent concentrations merit using micrograms per liter (l^{-1}). Other frequently used units are parts per million (ppm) and percent by weight. To avoid ambiguity in reporting results, significant figures are used, where all digits reported except for the last digit are known definitely.

9.5 Issues and improvements for the future

The science and art of water testing is highly advanced, as analytical instrumentation is available for detecting even parts-per-trillion concentrations of many contaminants. The speed and accuracy of these tests are outstanding. In many cases, the costs of these tests are high, but in view of regulatory requirements for testing at very low levels, there seems to be no reasonable way to reduce these costs. However, the regulatory climate as it exists in developed economies creates a need for lower testing costs. Therefore this is a major unmet need associated with water testing.

Another area of unmet need is in the identification of surfactants in textile wastewater. The effects of surfactants can be readily measured in terms of reduction of surface energy, foaming, aquatic toxicity, turbidity, and the like. However, it is often desirable to identify the exact concentration and identity of surfactants in wastewater. This is helpful, for example, in efforts to evaluate waste treatment system removal efficiency, or to reduce the detrimental effects of surfactants on the environment by pollution prevention (or 'cleaner production' as it is called outside the USA). At present, there is no reasonable scheme for surfactant identification in textile wastewater.

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10

Chemical analysis of colorants

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10.1 Introduction

In a single chapter of this type it is practically impossible to cover all of the techniques and methods that have been used, or even just those that are routinely being used, in analysing and evaluating colorants (and at the same time include information about colorants). This is especially true when these colorants may exist in a very wide range of sample types: pure dyes/pigments; commercially formulated colorants (different physical forms and different additives/impurities present); associated/incorporated with a substrate (textile, paper, leather, hair); or may be in a plastic, a cosmetic, a drug or a food product. However, whilst emphasis has been on the analysis of colorants used in textile coloration, some potentially relevant or interesting examples from other areas have been included to illustrate certain aspects or potential.

The general aim of this chapter is to focus on the major instrumental techniques that are used to analyse colorants associated with the textile coloration industries. The principal techniques that have been covered are spectroscopic techniques and chromatographic techniques. The capability and value of the techniques are highlighted by reference to relevant applications. Prior to focusing on the techniques, consideration is given to the nature of 'colorants' and also general issues, such as sampling, that are important in an overall analytical procedure. Section 10.2 on colorants includes some consideration of the health, safety and environmental considerations linked to colorants, which is a major driver for analysis of colorants. Note, in this chapter there has been a greater focus on the chemical nature, identity and quantitation of colorants (especially by instrumental methods) than on the property and measurement of 'colour'.

10.2 Colorants

'Colorant' is used as a catch all phrase to include any chemical that is coloured or can become coloured in a particular environment/under certain conditions and can be applied/used to impart the property of colour to an item. Dyes, pigments, lakes, toners and stains are all colorants.

A dye (or dyestuff, as they are sometimes referred to, especially in the USA) is a substance, nearly exclusively organic in nature, that is applied to a substrate in order to impart colour with some degree of permanence. At some point during application dyes are unimolecular, that is, they exist as individual molecules. Dye molecules often contain at least one water solubilising group, such as a sulphonic acid group, to aid their normal application route, which is usually from an aqueous medium. By far the main application area for dyes is in textile coloration.

The word pigment comes from the Latin 'pigmentum' meaning coloured material. A modern definition of a pigment would be: a substance consisting of small particles that is insoluble in the applied medium and is used primarily for its colouring properties. Pigments impart colour and some degree of hiding power (opacity) over the surface to which they are applied.

When it comes to defining lakes and toners, both of which are essentially specific types of organic pigment, there is a problem in that the accepted definition/understanding varies in different parts of the world. In the UK the following are accepted:

- A lake is an organic dye (originally natural dyes were used) that has been precipitated on to an inert (usually inorganic) substrate, e.g. alumina, to form an insoluble pigment; this gives a mixture of colorant and substrate.
- Toners can be anionic organic dyes, mainly acid dyes, precipitated as insoluble metal salts, e.g. barium or calcium. If the cation is lithium, potassium, sodium or ammonium the colorant is soluble and therefore a dye. However, salts produced with metals such as calcium, manganese, barium, strontium, and so on, are mainly insoluble. For this type of toner it is the anion that is the source of the colour. This is an easy method of producing relatively simple and economic pigments that find significant usage, especially in printing inks. Alternatively, cationic dyes (providing the coloured part of the molecule) can be precipitated with a complex inorganic acid (an anion), e.g. phosphotungstomolybdic acid. These toner pigments produce very strong, bright colours, but with poor fastness properties, limiting their use somewhat.

The term 'lake' is widely used in the USA, and elsewhere, to describe what are called 'toners' in the UK. To further add to the confusion with respect to American terminology, they often use the name 'Toner' for all organic pigments, as they 'tone' duller inorganic pigments.

A pigment powder consists of a mixture of crystals of different sizes (and often

slightly different shapes), agglomerates and aggregates that give an overall particle size distribution. This size distribution affects the properties of a pigment and can be measured by many methods, for example by microscopy. Some of the properties dependent on particle size distribution are surface area (which in turn affects dispersion properties of a pigment) and the pigment appearance. Particle size and shape greatly influence the amount of light reflected, scattered and absorbed by a pigment. When a pigment is highly scattering it is also very hiding (opaque).

Pigment powders are dispersed into a variety of media in order to impart colour and hiding power, for example, decorative paints, printing inks (for textiles as well as paper and packaging materials), coloured plastics and car paints. Dispersion is a difficult process and the presence of agglomerates and aggregates means some form of grinding must usually take place before the pigment can be used. Grinding is employed to break up large particles, reducing the average particle size (usually also narrowing the particle size distribution) and thus developing the colour strength properties of a pigment in a medium.

Light scattering properties of a pigment depend not only on particle size but also on the refractive indices of the pigment and the medium in which they are dispersed. Increased scattering occurs when the difference between the refractive indices of the pigment and the binder is maximised. Inorganic pigments tend to have high refractive indices (greater than 2) and are more highly scattering, resulting in an opaque appearance. Organic pigments have lower refractive indices and are less scattering and are, therefore, more transparent in appearance. It is also generally true that organic pigments have a smaller mean particle size (sub-micrometre range) than inorganic pigments (micrometre range). Changing the average particle size of a pigment can quite dramatically affect the performance properties (including appearance) of the product the pigment is a part of. For example the white inorganic pigment titanium dioxide (largest volume pigment, renowned for its high opacity) can be manufactured in a small particle size transparent grade that finds use as a UV protecting agent, for example in sun blocks. Additionally, a number of organic pigments have been manufactured to give special higher than normal mean particle size grades with different properties, such as higher opacities, compared to the equivalent pigments with smaller mean particle sizes. Thus particle sizing and particle characterisation techniques are very important for pigments (but are not considered in any detail in this chapter).

Other factors such as safety, cost and handling may also prove important in the choice of a pigment for a particular system. In addition, the pigment must be chemically compatible with the choice of medium, with any other pigments present and with any additives present.

For more detailed information on the chemistry, properties and applications of colorants the reader is advised to consult a suitable text book[1–10] and /or for the latest developments investigate the specialist journals related to colorants.[11–15]

10.2.1 Classification of colorants

There are thousands of different dyes and pigments (in terms of different chemical species) more than those used tens of thousands of years ago, for example from inorganic minerals used in cave paintings to new ones that are still being invented and investigated (for applications from dyeing textiles to those for use in medical treatments for diseases such as cancer). It is interesting to note that the vast majority of these colorants have been discovered over the last 150 years, since the advent of synthetically produced organic colorants following the discovery of mauvine by Perkin in 1856. The number of 'colorants' is multiplied further when different physical forms, different formulations and different suppliers (with often different quality products for basically the same colorant) are taken into account. When this is linked to the fact there are a wide range of uses and users of colorants, the importance of a classification system for colorants is clear.

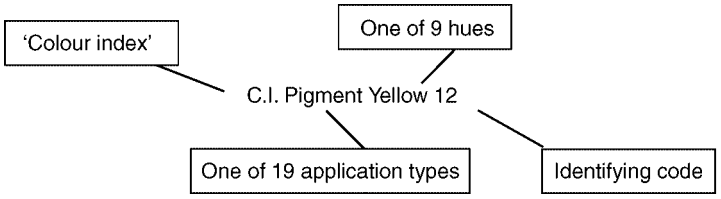
The classification system that is most commonly used for colorants is the one initially developed by the UK Dyer's Company (now transformed into the Society of Dyers and Colourists, SDC), with the first volume of the 'Colour Index' produced in 1924. More recently the Colour Index (CI) has been published on CD-ROM and as an on-line web version, which makes it much more manageable to access. It is now recognised as an exhaustive international classification system for dyes and pigments and is published by the Bradford (UK)-based Society of Dyers and Colourists in partnership with the American Association of Textile Chemists and Colourists (AATCC).

The Colour Index

Each colorant is assigned a C.I. Generic Name and a chemical constitution number (Fig. 10.1).

Generic Name: gives the nature of the product, the colour and a chronological number.

Chemical constitution number: a five figured number which is assigned to a colorant depending upon its chemical structure. Chemically similar colorants are given similar numbers, for example 77000–77996 are numbers of inorganic



10.1 Assignment of generic name to a colorant in the Colour Index.

Table 10.1 Usage categories classified in the Colour Index

Acid dyes	Food dyes	Reactive dyes
Azoic colouring matters	Ingrain dyes	Reducing agents
Basic dyes	Leather dyes	Solvent dyes
Developers	Mordant dyes	Sulphur dyes
Direct dyes	Natural dyes	Condense sulphur dyes
Disperse dyes	Oxidation dyes	Vat dyes
Fluorescent dyes	Pigments	

Table 10.2 Chemical classes classified in the Colour Index

Nitroso pigments	Triaryl methane	Sulphur
Nitro	Xanthene	Lactone
Azo–mono azo	Acridine	Amino ketone
Disazo	Quinoline	Hydroxy ketone
Trisazo	Methine	Anthraquinone
Polyazo	Thiazole	Indigoid
Azoic	Indamine	Phthalocyanine
Stilbene	Azine	Natural organic
Carotenoid	Oxazine	Oxidation bases
Diphenyl methane	Thiazine	Inorganic

pigments. Note, the aromatic structure (series of conjugated double bonds) of colorants was not represented properly in the early versions of the CI (this has been addressed in the latest CD-ROM and on-line versions).

For dyes, details of chemical class, structural formula (where disclosed), preparation methods, constitution number, chemical and physical properties, hue, dyeing properties on various substrates, printing properties, fastness properties, the C.I. Generic Name, references, patents and alternative names for the colorant along with commercial names (when notified by the colorant suppliers) are included. Details of non-textile applications are also provided. In the pigment section (of older versions) similar information is provided but the fastness properties are given as a detailed list of fastness in specific solvents and heat sensitivities. The traditional technical information about fastness properties was largely irrelevant because pigment fastness always depends on the medium in which it is being considered and providing such detailed information would have been fairly impossible. The newer versions give details of C.I. Generic Name, constitution number, structure, hue, chemical class, historical notes, CAS and EU numbers, commercial products, manufacturers, physical form of pigment, application areas and other comments. These details are all provided in one place for each pigment, making the newer versions much easier to use.

Colorants (especially dyes) are primarily classified based on their usage/application type and also on their chemical constitution (or chemical class) – see Tables 10.1 and 10.2.

The most common categories for pigment classification are simply based on chemical constitution:

- inorganic: the coloured oxides, sulphides, hydroxides, sulphates, carbonates, etc. of metals;
- organic: molecules based primarily on aromatic carbons for the backbone structure.

A further division can be made by splitting the pigments into groups depending on how they are obtained/produced:

- natural: pigments obtained from a natural source such as yellow ochre. Natural pigments are a minority group and used only for specialist applications;
- synthetic: pigments which are chemically manufactured. These are commercially the most important pigments.

Many of the naturally occurring pigments are preferentially obtained synthetically for good commercial reasons (especially for quality control of products). Further classifications of pigments can be made by splitting the pigments into colour groups, for example by classifying all red inorganic pigments into the same group. Organic pigments can also be further split by considering more detailed chemical constitution and structure.

Pigment manufacturers have been happy to support the colour index and provide information for its content. This stems from the fact that little industrial advantage can be gained by withholding pigment information and structures from the Colour Index, since most of the differentiation between chemically similar pigments comes about by manufacturing processes and after treatments, which are not revealed. The textile dye industry, on the other hand, has suffered quite badly in the past from 'non-traditional suppliers' using C.I. Generic Names for low quality dyestuffs. This has led to a reluctance to disclose dye information, particularly structures, to the Colour Index.

Colorants are supplied in a variety of physical forms: powder, liquid, presscake, paste, granules, master batch, chip or flake, flush colour or liquid dispersion.

10.2.2 Health, safety and environmental considerations[16–21]

Regulations that affect the colorant industries are numerous and over the last two decades or so they have generally increased in their potential to have an impact on manufacturers and processors of colorants; this is especially true for the more highly developed economies in the world. Companies have had to respond, in order to stay in business, and have generally been successful in doing so, but often after considerable expense (and often with continuing additional costs involved). Regulations can come from a variety of sources such as the European Union (usually in the form of a Directive), national regulations or even a regional or local authority. There are regulations that cover practically all areas of a company's

business and activities, including downstream aspects when chemicals leave one of their sites. Legislation is often based on the philosophies of 'prevention is better than cure' and the 'polluter pays'. This has clearly all resulted in the need for ever greater monitoring and analysis (and obviously not just in the various sectors of the coloration industry, but in all industries). This section will not attempt to cover all areas, but rather will try to focus on the major issues affecting the synthesis and handling of colorants.

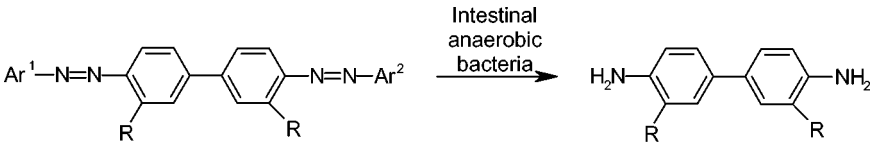
The complexity of the regulations, and the nature of the colorants industry, have caused, and still lead to, many difficulties for companies operating in the industry. Colorants are highly visible in the environment, especially synthetic dyes which can be seen in effluents at very low concentrations owing to their high colouring strengths. Commercial colorants have been designed and developed to possess a number of desirable application and performance properties, such as good fastness properties and high stability (as discussed in previous sections). It should therefore not be surprising to discover that, for example, dyes released in effluents can cause environmental problems since they are not generally biodegradable. Certain dyes are not effectively removed from treated effluents in traditional wastewater treatment plants. The relatively stable aromatic structural units which most dyes are primarily composed of are, at least in part, responsible for the specific environmental and toxic effects associated with the dyes. A number of studies have been carried out into the chemical and into the biochemical degradation of dyes and rely heavily on analytical techniques to study the kinetics of the processes or to aid identification of the products.

When considering the toxicological and ecological properties of pigments, the main factor to remember is that they have extreme insolubility in water and in the application media. Therefore, pigments (when pure) are generally considered to be physiologically and biologically inert.

As has already been mentioned, the most important chromophore system is that based on the azo group. This group is nearly always introduced into colorants via aromatic amines. Certain aromatic amines which were available (and frequently used) from the beginning of the synthetic dye era were later realised to entail considerable hazards and potential impacts. The acute toxicity of a few of these compounds was identified at a relatively early stage; the clinical symptoms that they gave rise to were called anilism. It has also been shown that, for example, benzidine-based azo dyes can be reduced to the free benzidine based diamine by intestinal bacteria[22] and also by UV light[23] (see Fig. 10.2).

Even today the legislation relating to colorants (manufacture, transportation, use, disposal) does vary a lot from one country to the next. Analysis of colorants often relates to checking that the sample does not contain restricted or banned species or will not break down to release any restricted or banned species. Recent German legislation on the use of azo colorants that have been produced from certain known or suspected carcinogenic aromatic amines will be discussed later.

At this point it is worth considering some well-known words of Paracelsus



10.2 Breakdown of benzidine-based azo dyes by intestinal bacteria to generate carcinogenic, free benzidines.

which, if you stop to think about for a while you may agree, are still relevant in modern times:

> 'What exists that is not poison? Every substance is a poison and nothing is no poison, it is merely the dose that makes a substance a poison'

Hence the need for extensive analysis!

ETAD (The Ecological and Toxicological Association of Dyes and Organic Pigment Manufacturers) was set up in 1974, as an international association, to minimise any possible negative impact of colorants on human health and the environment. The ETAD coordinate, and assist, the manufacturers of synthetic organic dyes and pigments with their ecological and toxicological efforts. There are about 46 member companies (based in 15 countries throughout four continents). Member companies are obliged to adhere to the ETAD Code of Ethics, which is based on the principles of responsible care. Further details regarding the ETAD and its activities are available from ETAD, PO Box 4005, Basel, Switzerland (www.etad.com).

Toxicity data

A survey of acute oral toxicity, as measured by the 50% lethal dose (LD_{50}) test, demonstrated that of 4461 colorants tested, only 44 had an $LD_{50} < 250$ mg kg^{-1} and that 3669 exhibited practically no acute toxicity ($LD_{50} > 5$ g kg^{-1}).[24] The rest fell somewhere between these two levels. The evaluation of these colorants by chemical classification revealed that the most toxic ones were found among the diazo (mostly benzidine derivatives) and the cationic dyes. It is widely known that some general cationic compounds have toxic properties. Pigments and vat dyes by comparison were discovered to have extremely low acute toxicity – presumably due to their insolubility/very low solubility in water and in lipophilic systems.

In another survey, summarised by Clarke and Anliker,[25] 3000 dyes in common use were tested for their toxicity to fish. The results showed that 98% of dyes tested had an LC_{50} value (lethal concentration to kill 50% of the test population) greater than 1 mg dm^{-3}. The LC_{50} value was of the order of 0.05 mg dm^{-3} for only 27 dyes (16 of them basic dyes of which 10 had triarylmethane structures), compared, for example, with DDT (a pesticide) at 0.006 mg dm^{-3} .

Toxicity associated with azo colorants

Three different mechanisms for azo dye toxicity have been identified and were reviewed by Brown and DeVito in 1993.[20] In order of decreasing number of published papers, these mechanisms are:

- azo dyes that are toxic only after reductive cleavage of the azo bond to produce aromatic amines (occurring mainly via anaerobic bacteria in the intestines). The aromatic amines are metabolically oxidised to reactive electrophilic species that are then able to bind to DNA;
- azo dyes with structures containing free aromatic amine groups that can be metabolically oxidised, without the need for azo reduction;
- azo dyes that may be activated via direct oxidation of the azo bond to highly reactive electrophilic diazonium salts ($Ar–N_2^+ X^-$).

Note, although it is believed that some bacteria can reduce any azo compound to aromatic amines, not all aromatic amines are toxic and hence not all azo colorants are potentially toxic. In fact, careful selection of intermediates during synthesis means that existing modern azo colorants are likely to be safe.

German ban on use of certain azo compounds in consumer goods

Most colorants are synthesised from aromatic amines and thus in some circumstances may potentially contain these amines as impurities or may, in some instances and under certain conditions, release aromatic amines if they are degraded in subsequent processes.

There has been much concern in the coloration industry since the mid-1990s regarding initially a proposed amendment to the German Consumer Goods Act and then subsequently its approval and implementation. The amendment, which was approved by the German Government in July 1994, placed a ban on consumer goods that contain azo dyes which could, through cleavage of one or more azo groups, form any of 20 specified aromatic amines known as the MAK III amines (see Table 10.3). These aromatic amines are listed by the German MAK Commission (MAK = maximum work place concentration) as either known human carcinogens (MAK III A1 list, compounds 1–4) or known animal carcinogens (MAK III A2 list, compounds 5–20). Although the potential risk to consumers from food and cosmetic products either containing or able to form MAK III amines is fairly self-evident, the toxic risk associated with dyed articles which will come into contact with skin is debatable, but seems likely to be extremely low. However, a cautious approach is surely preferable if human health may be at risk. It is obviously important that legislation is based on facts and sound scientific research so that safety can be ensured whilst at the same time unnecessary burdens are not placed on the coloration industries.

Azo pigments are generally exempted by the Fifth Amendment in the USA

Table 10.3 List of banned (MAK III) aromatic amines

	CAS-No.		CAS-No.
4-Aminodiphenyl	92-67-1	3,3'-Dimethoxybenzidine	119-90-4
Benzidine	92-87-5	3,3'-Dimethylbenzidine	119-93-7
2-Amino-5-chlorotoluene	95-69-2	2-Methoxy-5-methylaniline	120-71-8
2-Aminonaphthalene	91-59-8	3,3'-Dimethyl-4,4'-diaminodiphenylmethane	838-88-0
2-Aminoazotoluene	97-56-3	4,4'-Methylene-bis (2-chloroaniline)	101-14-4
2-Amino-4-nitrotoluene	99-55-8	4,4'-Oxydianiline	101-80-4
4-Chloroaniline	106-47-8	4,4'-Thiodianiline	139-65-1
4-Methoxy-*m*-phenylenediamine	615-05-4	2-Aminotoluene	95-53-4
4,4'-Diaminodiphenylmethane	101-77-9	2,4-Diaminotoluene	95-80-7
3,3'-Dichlorobenzidine	91-94-1	2,4,5-Trimethylaniline	137-17-7

owing to their extremely low solubility, which it is acknowledged means that they do not pose a risk to consumer health. However, some azo pigments are not exempted since they are sufficiently soluble, under the test conditions recommended, to yield detectable amounts of a listed aromatic amine. Note, in order to pass the test there must be less than 30 mg kg^{-1} (i.e. amount of amine present or released under the test conditions from 1 kg of the particular consumer item being tested) of each individual listed amine.

There have been concerns, within the coloration industry, regarding the actual analytical test procedures, since false positives (a result indicating a banned amine is present when the original colorant was not based on any banned amines) have been obtained with some colorants under the rather harsh sample treatment and extraction processes employed. The current official methods published do not use such harsh conditions. Perhaps the real winners in all of this are the contract analytical labs who do all the testing (and possibly the consumers to some extent)! Further details about the analysis of these species are contained in Section 10.6 on separation science.

Note, the German ban only restricts the use of about 5% of azo dyes (the rest are not based on listed/banned amines).

Dyes in effluents

The biggest issue for dyes is probably that of effluent treatment, since at the end of dye application significant amounts of colorant are usually discharged in an aqueous medium. The exact nature of the effluent will depend on the dye and the application method used. Generally reactive dyes are thought to cause the biggest potential problems. Dye effluents and their treatments are not considered in any great detail here, but the following issues are important:

- Presence of dyes in effluent: coloration power, high stability of dyes (wash +

light), chemical state of the dye (especially for reactive dyes) and very low biodegradability (filtration).
- Dyeing auxiliaries: acids, bases, salts, metal ions, surfactants, oxidising/reducing agents, dye impurities, high pollution load.
- Metal ions: very important for certain types of dyeing processes (especially wool), high toxicity but with respect to speciation.

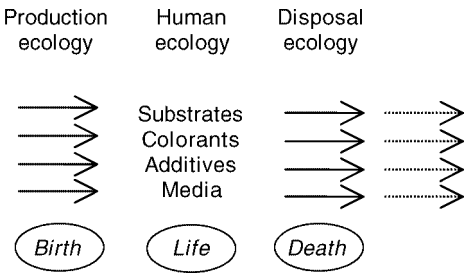
Pigment toxicity

The insolubility and excellent migration fastness of most organic pigments largely eliminate human health hazards. However, care may need to be taken when handling pigments owing to the potential presence of impurities (possibly heavy metals or residual amines). Good manufacturing procedures and appropriate sample clean up methods help to ensure that the levels of impurities are minimised. When pigments are incorporated into formulations it is invariably components other than pigments that are likely to pose the greatest ecological and toxicological risks. Atomic spectroscopy is normally used to test for the presence of heavy metals.

Inorganic pigments that are based on certain heavy metals in certain oxidation states (remember toxicity is dependent on physicochemical form, i.e. speciation) may need special consideration. For example, lead chromate pigments contain both lead and hexavalent chromium and, as a consequence, are defined by the EPA as carcinogenic. Experimentally, however, lead chromates have been found to be non-mutagenic and non-carcinogenic,[26] again presumably owing to their extremely low solubility. Note, the availability of potentially carcinogenic species will be partly dependent on the environmental conditions present (e.g. temperature, pH, etc.). Analytical methods based on atomic spectroscopy need to distinguish between 'available' and 'total' amounts of analytes of interest.

Life-cycle studies

If companies really want to be responsive to the health and safety needs of their workers and the users/consumers of their products and the welfare of the environment then life-cycle studies should be carried out for each product. The stages to be considered in a life cycle study are illustrated in Fig. 10.3. The creation (birth) and use (life) are of obvious concern to the producers, but should companies also have some responsibility for the safe disposal (death) of products when they reach the end of their useful life time? Can 'features' be designed/engineered into products when they are manufactured/formulated that will lessen any detrimental impacts on the environment when the products are disposed of, or could the components be recycled in some way? What happens to the constituents after disposal (life after death)? However, at the end of the day companies need to make money (and workers need to earn a living), therefore it can be argued that there



10.3 Key stages in life-cycle studies.

needs to be a suitable balance between ecology and economy. Analysis is important in the investigation of all of the stages of a life-cycle study in order to understand the species present and to aid the full evaluation of potential impacts.

In terms of the textile coloration industries, for example, investigations into all components – substrates, colorants, auxiliaries and media (likely to be water, but may be a binder-solvent system for a print paste) – need to be made. Additionally, any potential interactions between the different components in a product (e.g. dyed and chemically finished textile garment) or any degradation reactions that may lead to new species being formed (with potential new interactions and possible further species formed) should be considered. The fate of products after disposal has been of increasing concern over recent years. This is generally not an easy thing to investigate, owing to the long-term and complex nature of any breakdown of many products in the environment. For example, what happens to dyes when textiles are discarded? What happens to pigments when plastics degrade? This is an area where much has still to be investigated and learnt.

Conclusions

There are many issues associated with the safe manufacture, use and disposal of colorants and we have covered quite a few in this section. These are areas that will continue to be important, as legislation becomes ever stricter, so the role of analysis will remain important. There are other issues that have not really been covered in this section, but they are still important, for example:

- the special requirements of colorants used in food/cosmetics/pharmaceutical applications;
- the use of natural colorants and their associated problems;
- colorants used in products subjected to harsh conditions/environments (e.g. pigments in plastics that are processed at elevated temperatures).

10.3 General issues in analysis and the steps involved in analysis

Analysis and characterisation of materials is a key aspect of all manufacturing industries and many other industries. There are probably as many, if not more, reasons for carrying out analysis as there are different analytical techniques. Reasons for analysis include: checking the purity of, for example, raw materials, intermediates or finished products to see if they conform to specification; environmental considerations, for example to make sure discharge limits are not being broken – this may be a legal requirement; as a way of adding value to a product; to investigate the nature of a competitors product; trouble shooting when something goes wrong, and so on.

Analysis and characterisation of colorants involves many steps with a range of factors that need to be taken into consideration and not only during the actual analysis. It cannot be emphasised strongly enough that the key steps, in nearly all characterisation tests and analytical measurements, are those that occur before the sample goes anywhere near a characterisation/analytical instrument.

In dealing with materials evaluation, analysis and characterisation, it is worth considering a few general observations:

- The samples being evaluated should have a pedigree that suggests that the samples are worth the effort.
- All analysis and characterisation exercises produce results of one type or another. One major skill lies in being able to establish that the results can be relied upon. Another quality rests in having knowledgeable personnel who can interpret such results, putting them into a meaningful context, whereby appropriate conclusions can be drawn and action can be taken.
- Sample(s) acquisition and storage should be such that the results will never be compromised because of bad/inferior practices.
- The integrity of the samples is of importance. Much emphasis lies in the origin/history of the samples being analysed.
- Samples may have a composition that gives rise to complexities in both the analysis and the interpretation of results.

This chapter deals with these points prior to consideration of highly relevant analytical and characterisation techniques.

As has already been identified there are thousands of colorants and they may be mixed with, incorporated in, deposited on a wide range of different chemicals/materials. Thus there are a number of steps to go through before any actual and meaningful analysis can take place. These steps are considered further in the following Section 10.3.1 based on the approach of Skoog *et al.*,[27] however, just before that it is perhaps worth stating the general meaning of several common terms related to analysis, including ‘qualitative’ analysis versus ‘quantitative’ analysis.

Qualitative analysis: This can be considered in terms of the 'identification of the constituents of a sample without regard to their relative amounts'. Often it refers to elemental analysis, although it can refer to different chemicals within a mixture or even the identification of different functional groups (e.g. by infrared spectroscopy).

Quantitative analysis: Here the 'identification of the relative amounts of substances making up a sample or establishing the amount of one particular compound/element in a sample' is important. Quantitative analysis often refers to elemental analysis, but it may refer to any constituent of the sample.

Accuracy: Refers to the closeness of the agreement between the result of a measurement or analysis and the true value that should have been obtained from the measurement or analysis.

Precision: Refers to the closeness of the agreement between independent test results obtained under stated and closely controlled conditions. Note that precision depends only on the distribution of random errors and does not relate to the actual true value. 'Independent test result' means that each result is obtained in a manner that is not influenced by any previous result.

It is important to remember the terms 'precision' and 'accuracy' are not interchangeable – they mean quite different things.

10.3.1 Stages involved in successful quantitative analysis

The first task in the analysis or characterisation of any sample is to consider the requirements. Why is the sample being tested and what information is actually required? There can be many reasons for carrying out analysis (as discussed at the start of this section) and these reasons can influence the technique used for the analysis, the method selected and the way the test is actually carried out. When considering the requirements and selecting the method it will normally be necessary to take into account a variety of issues including the following:

- the number of different analytes in (or properties of) the sample about which information is required (and whether simultaneous or sequential analysis is possible by just one technique);
- the nature of the analyte(s) of interest;
- the sample matrix (this may help or hinder the analysis);
- the number and frequency of samples;
- the availability of suitable standards (if required);
- the existence of a suitable method(s) (either in-house or in the literature);
- the accuracy and precision needed;
- the urgency and timescale (related to time needed for sample preparation and analysis);
- the equipment available;
- the expertise available;

- the reliability needed;
- the need for more than one technique to be used to increase confidence in the results;
- the type of validation required;
- the full cost involved in the analysis (including sample preparation and staff time).

A number of these points may lead to consideration of whether the analysis should be carried out in-house or sent out externally on a contract basis.

Having decided on a method the next step should be to obtain a representative sample. If you work in an analysis laboratory then it is likely that samples will just be passed on to you, but in many cases it will be worth enquiring (or offering advice) about sample collection. The importance of sampling can be appreciated when you consider that the possible fate of many tons of product is determined based on the results of tests on perhaps just milligrams (mg) of a sample or that the pollution level of a river may be determined by tests on just a few millilitres (ml) or even microlitres (μl) of a sample or that the quality of a batch of thousands of leaflets may be based on maybe just one section of one leaflet. Thus, the sample used for analysis must be representative of the whole (whatever that may be) and the sample should be homogenous. It could well be that the best approach to take would be to obtain a number of samples, from different places in the bulk, and either analyse them individually (to check uniformity) or blend them together to obtain a single representative sample for analysis. The number and selection of samples should be carried out using a suitable statistical basis. You also need to consider whether the sampling process could affect the composition, for example differential adsorption in the sampling system.

Having obtained (or been provided with) a representative sample the next stage will be to prepare the laboratory sample. There are again a number of points to consider, such as will the sample go off (decompose) or change over a period of time or under certain conditions (for example , the hydrolysis of reactive dyes); and does the sample absorb water from the environment (especially important where accurate weighings are required) as this may affect the results obtained by various techniques?

To increase confidence in the results a number of replicates will be needed. You will need to consider how many repeats will be suitable based on the technique and the level of accuracy required. The replicate samples should all be prepared from the start and should not be, for example, just a variety of dilutions from a single stock solution. Any inaccuracy in the initial weighing or making up of the stock solution will lead to subsequent dilutions also being inaccurate (although the precision may be good). Remember accuracy and precision do not mean the same thing!

For performance evaluation it will be important to maintain the physical integrity of each sample prior to testing. Some laboratory samples will need to be

prepared so that they are in the most suitable physical form for the analysis. In many cases this will mean that samples (which are often solids) will need to be dissolved in a suitable solvent. The solvent needs to be suitable not only from the point of view that it can satisfactorily dissolve all the analytes of interest, but that it can be successfully used with the technique being employed for the analysis/characterisation required. Therefore, selected solvents should not interfere (or react) with the sample and should not give a response during the analysis that will in any way affect the response(s) from the analyte(s). For example, certain organic solvents will absorb electromagnetic radiation of the same wavelength as certain analytes and therefore overlapping bands will appear in some cases with techniques such as ultraviolet (UV) and infrared (IR) spectroscopy leading to inaccuracies and/or confusion. Any reagents or solvents used must be of an appropriate quality for the work being carried out; this often means that fresh, high purity materials (which are expensive) are required, in order to avoid the potential confusion/inaccuracies that may be caused by contaminants.

Depending on the analyte(s) of interest and the matrix present, the elimination of interferences could be an essential step in obtaining acceptable results. There is a wide range of techniques available for eliminating interferences, usually aimed at cleaning up the sample and removing the unwanted species or removing the analyte(s) of interest and leaving the other components behind. Eliminating interferences may be part of the sample preparation technique or it could be an automatic feature of a particular technique or instrument. For example, with gas chromatography (GC) only components which are volatile at the injection temperature will enter and be potentially separated in the GC column, additionally many instrumental techniques are available with detectors that are selective (or can be made selective) in what they will actually detect.

For quantitative analysis, especially where accuracy and reproducibility (possibly from month to month or even year to year) are very important, calibration and validation are essential. Calibration of an instrument may be an in-built feature of an instrument (e.g. the internal calibration of the frequency scale of a Fourier transform infrared (FT-IR) instrument using an in-built laser) or may require regular external checking with suitable certified reference materials. When the unknown concentration of a particular analyte in a sample has to be established, a calibration graph (over a suitable concentration range) will need to be produced for the analyte concerned using standards (preferably certified reference materials) of known concentrations. Work may need to be carried out to validate the complete method, including all sample preparation stages and instrumental measurements, and should investigate any effects caused by the sample matrix. Note that an analyte present in deionised water (e.g. a standard) may not give the same response on an instrument as the same analyte at the same concentration when present in a salt solution or aqueous dispersion (e.g. a sample).

When the sample has been prepared properly and the instrument has been set up correctly (check and make a note of all manually set instrument settings, for future

reference or to make sure that none of the instrument variables have been changed since the last time the same type of analysis was carried out) the same should be ready to be analysed/characterised. Then measure a property of the analyte, for example how much electromagnetic radiation it absorbs at a particular wavelength (see Section 10.4), or how much energy it takes in as it is heated (as in thermal analysis). This is what most people would consider to be the actual analysis step, although it can be argued that analysis involves a lot more than simply measuring some property of an analyte. This stage can also be considered as the data acquisition step (where the raw data about a sample is obtained).

After obtaining the raw data (i.e. the original data) for a sample it is nearly always necessary to carry out some data manipulation (this is usually carried out by the instrument or an attached data processor, e.g. a computer) which may include making corrections or calculating concentrations based on calibration work carried out earlier. This stage gets the data into some useful form that is easily recognised and can be used by the analyst.

The results from the analysis/characterisation of a sample may be presented in many different formats, depending on the instrumental technique used and any associated software used for handling the data, including chromatograms, spectra, thermograms, elemental maps, micrographs, tables of data and a single numerical value. In modern times the interpretation of the data/results generated has become easier (and is sometimes carried out automatically by instruments/computers) owing to increases in the speed, power and sophistication of computers and computer software. Having said that, care still needs to be exercised when interpreting or analysing results – always ask yourself if the results and their interpretations make sense – mistakes can be made (e.g. in preparing samples or standards, or in setting up the instrument) and there is a danger that because the information comes from a computer it is automatically assumed to be correct.

Following the interpretation of the data it should be possible to write a report based on the findings. To be truly meaningful and useful the results should report the confidence levels, taking into account the errors (calculated from replicate samples), associated with any values reported. Additionally, units (where relevant) must be included for all values quoted. In most cases it will also be necessary to include the following information:

- the unique sample name/number;
- the experimental method used or reference to a standard method if used (for sample preparation and for analysis);
- instrumental type (and unique identifying label if more than one in the same laboratory) and operational settings;
- the date, place and name of the analyst associated with the work.

Note that operator effects on results obtained can be significant when sample preparation and analysis are not fully automated; this is true even when a standard method is being followed.

You should bear in mind the points made in this section when reading all the other sections. Remember there may not be much point in measuring and reporting results, for example, to three decimal places just because the instrument/computer generates them that way, especially if there are or could be gross errors in your sampling or sample preparation techniques.

Commercial colorants, inks and surface coating formulations are multi-component systems that have generally increased in their chemical complexity owing to the pressures continually to improve formulations and their performance (in a highly competitive market), together with the ever stricter health and safety legislation which is being introduced. Heterogeneous samples such as dyed textiles also present particular potential problems when it comes to analysis.

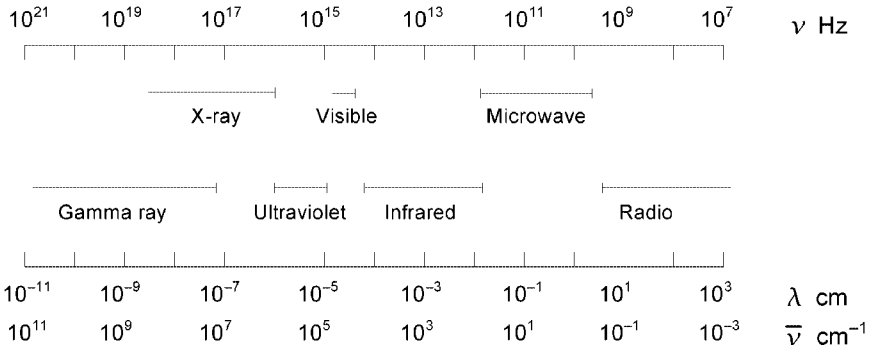
10.3.2 Summary

- Before starting (or requesting) an analysis it is important to consider carefully exactly what information is required and what level of uncertainty (i.e. errors) is acceptable as this will have a big influence on the way the sample is handled and the technique(s) employed.
- There are a number of different steps involved in characterising or analysing a sample – all steps are important to some extent and can influence the result obtained.
- A representative sample must be obtained and used – the consequences of failing to do this could be enormous as wrong decisions could be made based on the results obtained.
- Care with sample preparation is essential especially if quantitative results are required.
- All conditions and instrument parameters should be recorded so that a test could be repeated if required, in exactly the same way, at a later stage.
- Where possible quote the errors and the units associated with any values reported as the results of an analysis or characterisation.

10.4 Molecular spectroscopy/spectrometry

Spectroscopy is the study of the interactions between radiant energy and matter. The frequencies, wavelengths and wavenumbers associated with different regions of the electromagnetic spectrum are shown in Fig. 10.4.

This is of fundamental importance to chemists and materials scientists as the wavelengths at which an organic compound absorbs radiant energy are dependent upon the structure of the compound concerned. Therefore, spectroscopic techniques may be used to determine the structures of unknown compounds and to study the bonding characteristics of known compounds. It must, however, be remembered that most 'real' samples contain a number of components and hence the spectral data obtained, which is dependent on a number of factors, including how it is collected, is likely to contain 'contributions' from the various



10.4 Regions of the electromagnetic spectrum.

components. Thus, analysing mixtures can lead to problems of interference such as overlapping absorbance bands. However, with experience and the advances that have been made in developing the software packages (that are an integral part of most analytical instruments) data processing and manipulation can assist in the extraction and understanding of a far greater amount of chemical information from mixtures using modern technology and methods. It should be noted that impurities or even analytes of interest within a sample (especially compounds present at low concentrations), unless they contain a particular chemical group (usually needing to be unique to a single chemical within the sample) that interacts (e.g. absorbs a specific wavelength) very strongly, can escape detection on examination by molecular spectroscopy.

Whilst spectroscopy techniques can be used on their own to obtain spectral information about a sample they are also commonly incorporated as a detector as part of another technique, for example the use of an ultraviolet absorbance detector as part of a liquid chromatography system. In recent years there has been much enthusiasm for the research and development of 'hyphenated' techniques, that is the interfacing/linking together of two or more techniques, because of the enhanced additional data that can be generated.

10.4.1 Infrared (IR)

The region of the infrared normally employed for the analysis of materials is in the wavelength range from 2.5×10^{-6} m to 16×10^{-6} m; this is more commonly expressed as the number of waves per cm, i.e. 4000–625 cm^{-1} (in wavenumber units). This region falls within what is often referred to as the mid-infrared region. The full IR region is normally regarded as covering wavelengths from about 750 nm up to 1mm. There has been increasing interest in the near infrared (NIR) region in recent years for process monitoring and quality control (QC) checks of materials, for example, when they enter or leave a site.

The 'fingerprint region' contains numerous and frequently overlapping absorption bands, the exact assignments of which are normally impossible even when a compound of known structure is analysed. The term fingerprint is employed because the pattern of absorption in that part of the spectrum is uniquely characteristic of the compound concerned. The main value of this region is in establishing the identity of samples obtained from different sources. If two samples are identical then their infrared spectra will be exactly superimposable when measured under the same conditions.

It should always be remembered that an infrared spectrum for a mixture of organic compounds will contain contributions (bands that will often overlap) from each of the components present in the mixture. Therefore, interpretation of spectra obtained from mixtures can be very difficult, especially if any or all of the compounds are unknown. It should be noted that impurities (compounds usually present at low concentrations), unless they contain a very strongly absorbing group, can escape detection on examination of infrared spectra.

Infrared instruments

Calibration of instruments

It is good practice to check the accuracy of infrared spectrophotometers (including FT-IR spectrophotometers) at regular intervals by reference to the infrared spectrum of a standard. Conventionally polystyrene has been employed for the purpose (the bands at 1603 and 1028 cm^{-1} being particularly useful). The frequency accuracy of a modern instrument is normally internally calibrated automatically by the instrument.

Evolution of FT-IR technology

Fourier transform infrared (FT-IR) spectroscopy is now one of the most popular techniques in analytical chemistry, this technology having several advantages compared to conventional dispersive infrared instruments. Developments in instrument hardware, in computer software (usually by the instrument manufacturers) and in computing power generally has resulted in very powerful data collection and data handling systems for the analysis and characterisation of all sorts of materials including colorants.

Modern times

Specialised sampling techniques such as attenuated total reflectance (ATR) and diffuse reflectance (DR) have been found to be extremely effective and hence have gained considerable popularity. Microsampling, for measuring very small samples, has become a common technique over the last decade as beam condensers and infrared microscopes (plus accessories) have been improved.

Applications of FT-IR

The physical state of a sample and the information required has to be considered when deciding how best to carry out an infrared analysis of a sample. As has been mentioned previously, sample preparation can be very important and there are examples where this is true for colorants analysed by infrared. For example, if the polymorphism (capable of existing in more than one crystal form) of a colorant is to be studied, then the sample preparation step(s) should not physically nor chemically alter the sample, that is, minimal and mild sample preparation should be used (ruling out the use of the alkali metal halide disk technique, where grinding can cause conversion in crystal forms).

Identification of unknowns

One of the most frequent ways in which infrared spectroscopy is employed is as a qualitative tool for the identification of 'unknown' materials. This is because the absorption bands that a polymer or other material has are very characteristic and reproducible for the chemical functional groups/components within that material. A spectrum may be considered to be a 'fingerprint' for a sample, particularly the region 1400–600 cm^{-1} which is commonly termed the 'fingerprint' region (described briefly earlier in this section). To be a certain material, without any doubts regarding the exact identity, a sample spectrum needs to be in agreement in all respects with a reference spectrum. Thus, all the bands of the reference spectrum need to be present and each band should have the same breadth, that is cover the same frequency range as the reference spectrum. The relative absorbance of each IR band to the others should also agree. If there are additional bands not present in the reference spectrum then the sample material may be a mixture or have a slightly different structure to the reference material. If the match is perfect, it provides a very good basis for identification. It should be remembered, however, that there is always the possibility that a material is a mixture in which the individual substances absorb in common ways. This would be the case for a mixture of polyethylene and paraffin wax. Therefore, other techniques such as thermal analysis may be needed to detect the mixture or confirm the purity of a sample.

Approaches to identifying unknowns: There are a number of different approaches that may be taken to identify unknown compounds from their spectra, the most common ways being:

- frequency–structure correlation charts;
- flow charts based on principal infrared bands;
- collections of reference spectra (these may be stored either in a book or on a computer).

The use of correlation charts can help in piecing together an unknown's identity. For example, carbonyl groups absorb infrared energy over a certain range within

which aldehydes, ketones, carboxyls and other forms of carbonyls are known to absorb at specific frequencies. Although it may not necessarily be possible to identify an entire compound (e.g. a specific dye), it is feasible to identify functional groups based on absorptions in particular regions, for example the carbonyl region, and to which the wavelength corresponds, for example an ester functional grouping. Although it may not be possible to identify a material completely, the spectrum will usually provide an indication of the kind of chemical structure that is present.

Brief details of IR techniques/methods are included in this section. For a more comprehensive explanation of the principles, practicalities and a wide range of applications readers are advised to refer to the excellent book by Chalmers and Dent.[28]

Quantitative analysis of mixtures

Infrared analysis can potentially determine quantitatively the relative amount of different components in a system, such as a mixture of two dyes. The general requirements are that for each component there should be an absorbance band that is relatively sharp and that it should be unique to the component that produces it in the system in which it exists. It is important that sample thickness/concentration (depends on how the samples are prepared and mounted) is adjusted so that absorbance is in a linear range relative to concentration, that is, absorbance should not be too high.

The mass (m) ratio of components is directly proportional to the absorbance ratio of the bands corresponding to each component. Thus for a mixture of two colorants (colorant A with a unique band at 1720 cm^{-1} and colorant B with a unique band at 2235 cm^{-1}) the following can be used:

$$\frac{\text{Concentration of A (m/m)}}{\text{Concentration of B (m/m)}} = K \frac{\text{Absorbance at 1720 cm}^{-1}}{\text{Absorbance at 2235 cm}^{-1}}$$

A common procedure is to produce standards of known composition and then plot the absorbance ratio against the mass percentage of one component or against the mass ratio. The plot should be linear and pass through the origin, that is, the absorbance ratio is zero when one of the components is absent. The constant K is calculated from the slope of the plot. The mass percentage of an unknown may be determined from the graph using the absorbance ratio or directly by calculation using the previously obtained value for K.

For the most accurate quantitative analysis, homogenous samples must be used and care needs to be taken when preparing the samples – dependent partly on the mode of operation. This may involve preparing solutions, however, this requires a solvent whose spectrum does not interfere with the required bands of the analytes in the sample.

Modes of operation

Transmission techniques

When measuring transmission for thin samples, for example films, there is a need to ensure absorption of IR energy is not too strong in any part of the spectrum. For a colorant in a powdered form the traditional way to obtain an infrared spectrum is to dilute the colorant whilst creating a homogenous dispersion either in Nujol (a liquid paraffin) or an alkali-metal halide (normally potassium bromide). Clearly, it is important that the diluent (e.g. Nujol or KBr) does not have absorption bands in the same region of the spectrum as the sample being analysed.

To make a KBr disk the sample is usually pre-ground before mixing (in a ratio of about 1:200–100, depending on the strength of the infrared absorption of the sample) with dried potassium bromide. Following mixing to form a homogenous material the powder is transferred and appropriately packed in a die assembly and then placed in a hydraulic press. A load of about 10 tons (10 000 kg) is normally applied. The resultant disk should be translucent with the colorant homogenously distributed and can be carefully (to avoid contamination, e.g. moisture and grease from fingers, and damage) positioned in the disk holder that is then placed in the infrared instrument ready for data collection.

Reflection techniques

For some sample types, for example a coated substrate, it is not possible to collect an infrared transmission spectrum, whereas in some cases (e.g. when there are concerns over the effects of sample preparation) it may be more desirable to collect a reflected spectrum. The most popular reflection techniques nowadays are internal reflection spectroscopy (IRS) and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS)

DRIFTS: Solids such as powders and fabrics can be studied by collecting the diffusely scattered radiation, without the need for any major preparation of the sample. In reality what happens is that some radiation travels (i.e. is transmitted) through the particles (in the case of a powder) where characteristic wavelengths for the material concerned are absorbed before being scattered at any angle. The infrared spectrum obtained from a sample in a DRIFTS accessory can be more complex than a traditional transmission spectrum, however, because a number of inter-actions and processes happen to the radiation (transmission, absorption, specularly reflected, internally reflected and diffusely scattered). Again the quality of the spectrum obtained for a sample will be dependent on a number of factors including the experimental conditions used (e.g. DRIFTS accessory employed) and sample properties such as homogeneity – both physical (e.g. particle size) and chemical; concentration and packing density (for powders it may be necessary to dilute them with an alkali-metal halide); particle size; refractive index of sample; surface rugosity; and absorption coefficients.

ATR: Internal reflection spectroscopy is sometimes called multiple internal reflectance (MIR), although it is generally better known as attenuated total reflectance (ATR). A further name that has been used is total frustrated reflection (TFR). A variety of geometries and of ATR crystal materials have been utilised in the development of a range of ATR accessories for infrared instruments. These differences result in a wider range of applications and even in differences in the data that can be obtained for a given sample, for example, owing to differences in the refractive indices of different crystal materials the depth of 'sampling' for a given material will depend on the crystal used. The angle of incidence of the infrared beam at the crystal–sample interface also influences the 'sampling' depth. Analysts can sometimes use this phenomenon to their advantage, for example when studying surface coatings/modifications (by using a range of crystal materials and/or angles of incidence).

A potential advantage, that many users utilise, with ATR accessories is that often minimal or no sample preparation is required. Whilst an infrared transmission spectrum of a given colorant will have absorption bands in the same position as an infrared ATR spectrum of the colorant the relative intensities of the bands will vary. The explanation for this phenomenon is again related to the 'sampling' depth, which is actually wavelength-dependent in ATR experiments with the bands becoming relatively stronger with decreasing wavenumber (i.e. with increasing wavelength). Thus, ATR spectra are not equal energy spectra!

FT-IR microscopy

The FT-IR microscope combines microscopy with IR spectroscopy to provide a versatile instrument for molecular microanalysis. The technique has really taken off in the last decade and has embraced a wide range of applications. Nowadays, developments in PC and software products allow for instruments with remote control (including focusing) of microscopes.

Application areas for FT-IR microscopy include:

- composition of plastic laminates (it is necessary to prepare a cross-section of the laminate by conventional microtomy);
- *in situ* analysis of surfaces – particularly well suited for investigating surface coatings and contaminated films;
- characterisation of crystalline substances (including individual particles – if they are not too small);
- analysing evidence in forensic investigations, e.g. fibres or paint chips from the suspect/scene of the crime;
- identification of fibres.

Table 10.4 General comparison of spectroscopic techniques

Infrared	Raman
Requires functional groups to exist which are capable of forming dipoles	Requires functional groups to exist which are not generally capable of forming dipoles
i.e. asymmetric bonds	i.e. symmetrical bonds
Examples of IR active bonds are: >C=O, >N–H, –O–H, S=O, –C≡N	Examples of Raman active bonds are: –C≡C–, >C=C<, C–S, –S–S–, –N=N–, –S–H

10.4.2 Raman

Raman spectroscopy is by no means a new technique, although it is not as widely known or used by chemists as the related technique of infrared spectroscopy. However, following developments in the instrumentation over the last 20 years or so Raman spectroscopy appears to be having something of a rebirth. Raman, like infrared, may be employed for qualitative analysis, molecular structure determination, functional group identification, comparison of various physical properties such as crystallinity, studies of molecular interaction and determination of thermodynamic properties.

Raman spectroscopy is a light scattering method that is non-intrusive; sampling, sample form and sample size are generally not restrictions for the analysis. Like infrared, it can be employed equally successfully for the analysis of solids, liquids and gases.

The reason why Raman spectroscopy has never really been widely practised, certainly nowhere near as much as infrared spectroscopy, is because a large proportion of samples either fluoresce or contain impurities that fluoresce or the samples 'burn out' when excited by the visible lasers commonly employed in conventional Raman measurements; this has been especially true for colorants. The origins of this fluorescence and the thermal heating comes from electronic transitions occurring in samples at or near the laser frequency; the fluorescence is generally many times stronger than the Raman scattering signals and thus swamps out the Raman signals in a spectrum.

When considering Raman spectroscopy it is useful to compare it to infrared spectroscopy since the two techniques are often considered complementary. The selection rules for the two techniques can be stated simply as in Table 10.4.

Strengths and potential advantages of Raman spectroscopy

Raman spectroscopy actually has quite a few benefits and can for certain samples be a better technique to use than infrared spectroscopy. The major strengths of Raman spectroscopy as an analytical technique are:

- Water is only a weak Raman scatterer, hence analyses in aqueous solutions are possible.
- Glass is only a very weak Raman scatterer, hence samples can be analysed in glass vessels (useful for volatile and/or toxic samples).
- Analysis is easy (plus no/minimal sample preparation) – this is often also true with FT-IR analysis (depending on the mode of operation).
- Samples may be in any physical state and investigated under a range of temperatures and pressures.
- Only small quantities are required and Raman microscopes are available (with greater resolution than FT-IR microscopes and even greater resolution as the excitation wavelength from the laser becomes shorter.
- Raman optics do not need to be purged since CO_2 and H_2O, generally, do not interfere with Raman spectra unlike in infrared spectra (this is not such an issue as modern software can often compensate for this phenomenon).
- Investigation of low wavenumber bands is possible compared with infrared which has a much higher wavenumber cut-off (for infrared this is variable and dependent on the mode and infrared accessory used).
- Raman bands are sharp and there are fewer of them, hence interpretation can be easier;
- Raman scattering intensity is linearly related to concentration.

Developments in Raman spectroscopy, with applications for colorants, have included resonance Raman, surface enhanced Raman spectroscopy (SERS), surface enhanced resonance Raman spectroscopy (SERRS) and near-infrared Fourier transform Raman spectroscopy (NIR-FT-Raman), with the latter technique discussed in the next section.

Near infrared Fourier transform-Raman spectroscopy

This technique has emerged and developed within the last 15 years or so. A Nd:YAG near infrared laser is normally used that emits its energy at 1064 nm (i.e. in the NIR); this is a much higher wavelength than in conventional Raman spectroscopy (which uses lasers that emit in the visible region). The major advantage of this is that there is a reduced (but not totally eliminated) problem of fluorescence, that is, fewer samples fluoresce. A potential disadvantage may be a reduction in the sensitivity of the detector used.

Advantages of the Fourier transform technique

Many of the advantages of Fourier transform infrared (FT-IR) over dispersive infrared also apply to FT-Raman over conventional Raman, for example signal averaging and spectral subtractions, together with all frequencies are measured simultaneously and hence there is increased speed of analysis.

Use of NIR energy as the mode of information transport provides the potential for high signal throughput and the use of fibre optic cables over long distances for

remote on-line analysis; this is not a viable option for mid-infrared analysis since there are not the materials available which could carry the mid-IR signals unaltered over long distances.

Applications of Raman spectroscopy

The application areas are as varied and numerous as those found in infrared spectroscopy, however, the technique has not been employed quite so widely (due largely to the cost of the instruments, the problems of fluorescence and 'burn out' mentioned earlier, and the time taken by older instruments to obtain a reasonably good quality spectrum).

In addition to being capable of helping in the elucidation of the structure of a colorant, Raman spectroscopy can be used to monitor organic reactions in aqueous media, such as the diazotisation of an aromatic amine, the coupling reaction and the condensation reaction in the synthesis of a reactive azo dye.[29,30] It is interesting to note that the preparation of samples as an IR KBr disk, or for very sensitive, highly coloured or strongly absorbing samples as IR Nujol mulls between salt flats, can give good strong Raman spectra even for some black azo dyes that burn out when analysed as the neat powder (as illustrated by Chalmers and Dent[28]). Applicability and difficulties with some phthalocyanines (linked to fluorescence caused by transition metals such as copper) have been investigated.[31] Additionally, obtaining spectra of colorants whilst they are in a polymeric matrix (such as dyes on textiles) is possible, for example, with coloured cellulose film, since cellulose is a very weak Raman scatterer.[28] Raman spectroscopy is also widely used for many polymer systems (from fibres to films, to paint systems).

10.4.3 Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) spectroscopy is an important analytical technique for an organic chemist for the characterisation of molecular structure and may be used for samples in the liquid or solid state. NMR has been used traditionally to provide a map of the carbon/hydrogen framework of a chemical. Many nuclei behave as if they are spinning about an axis and because they are charged appear to act as bar magnets. The two most important nuclei that act in this way are ^{1}H and ^{13}C. Examples of other nuclei that are sometimes utilised, and are present in the structures of some colorants, include ^{19}F and ^{15}N. NMR and IR are often used to complement one another. Again though, the technique works best for relatively pure, single component, samples.

Fourier transform can be used with NMR experiments and allows simultaneous irradiation at different frequencies to be performed. This is controlled by a computer which does the mathematical calculation (transformation) to convert the signals obtained into a spectrum. This means multiple scans can be carried out much faster. Typically four scans are used for ^{1}H, while typically 1000–10 000

scans are averaged for ^{13}C (to reduce noise, i.e. increase the signal to noise ratio). Solvents will also give peaks (these must be away from the region of interest). The use of deuterated (^{2}H) solvents, such as D_2O, to avoid the appearance of ^{1}H peaks from the solvent in ^{1}H-NMR is common practice. It is now possible to use NMR in a variety of ways (including two-dimensional NMR) to obtain all sorts of information.

Applications of NMR

NMR is a firmly established technique, however, its full analytical potential has clearly not, as yet, been fully realised with new applications and experiments carried out by this method still being regularly reported.

Solid state NMR

The early investigations with NMR by physicists in the late 1940s and the 1950s, examined both solutions and solids extensively. When chemists adopted the technique – and transformed NMR into one of the most powerful methods for the characterisation of compounds – only solutions and liquids were studied, with few exceptions (between 1956 and 1976). However, in modern times it has become accepted that solids are (almost) as amenable to NMR as solutions.

In isotropic solutions of low viscosity, linewidths for typical spin = ½ nuclei, such as ^{1}H and ^{13}C, are substantially less than 1 Hz (i.e. very narrow). Crystalline samples, such as pigments, and rigid amorphous materials, however, usually exhibit such extreme broadening that all the information on chemical shifts and coupling constants appears to be lost. Carbon-13 and proton NMR linewidths for typical organic solids in powdered or microcrystalline form are roughly 30 kHz and 60 kHz respectively – hence the lack of interest by chemists 20–30 years ago. The chemical information content of solid state NMR is, however, far greater than that of solutions, the problem is extracting it.

The reasons for the complexity of solid state NMR spectra are:

- Orientation dependence of the NMR interactions which give the chemical information.
- Severely restricted mobility of molecules in solids so that little averaging can take place; in contrast, in solution molecules 'tumble' rapidly, isotropically and chaotically, at a sufficient rate that the NMR parameters are averaged to their isotropic values.

Magic-angle spinning (MAS)

What is required for solids is obviously the equivalent of the molecular tumbling in liquids and solutions. The approach normally employed is to spin a bulk sample coherently about an axis making an angle, β. If β is set at 54° 44', all anisotropy

effects are removed from the spectrum, and each powder pattern will collapse to a single line at a frequency governed by the relevant isotropic chemical shift.

Although in principle MAS should be effective for obtaining spectra of dilute spins (including ^{13}C) in the presence of abundant spins (such as ^{1}H or ^{19}F), in practice the usually available spinning speeds are inadequate. Instead, double resonance (decoupling) techniques, as often employed in solution state NMR, are used. The powers necessary for solids are orders of magnitude greater than for solutions. This high-power proton decoupling (HPPD) technique combined with MAS, provides high-resolution spectra of dilute spins (e.g. ^{13}C) for most solids.

Other NMR modes of operation include:

Cross-polarisation (CP), which involves a cross-pulsing sequence. Under the appropriate conditions, magnetisation flows from protons to ^{13}C during the contact time, when resonant radio frequencies are applied to both protons and the nuclei to be observed (e.g. ^{13}C).

Combined rotation and multiple-pulse spectroscopy (CRAMPS). A special pulse sequence, in addition to MAS, is required for high-resolution proton NMR in solids. This technique is known as CRAMPS.

10.4.4 Ultraviolet (UV)/visible (vis) spectrophotometry

Ultraviolet and visible spectrophotometry is usually carried out with solutions for the quantitative determination of components that absorb in the UV or visible regions of the electromagnetic spectrum. Solvents must be selected that do not absorb in the region of interest. The usual region of absorbance that is of interest is within the range 180–780 nm and is associated with electronic transitions in double bonds (e.g. the carbonyl (>C=O) group at ~280 nm or a benzene ring group at ~250 nm); the linking of two aromatic groups, such as benzene rings, through an azo (–N=N–) results in a bathochromic shift (to longer wavelength) into the visible region. Some spectrophotometers extend their wavelength range significantly beyond 700 nm and cover the near-infrared, where some colorants (often specialist ones) absorb electromagnetic radiation.

A suitable detector measures the percentage transmission, which can be converted to an absorbance value for quantitative analysis, for example, in quality control for the quantitative determination of the strength of a particular dye. Calibration with solutions of known compositions and concentrations are normally required so that the concentration of a dye solution of unknown concentration (e.g. a residual dyebath or wash-off solution) can be determined; this is possible through the use of the Beer–Lambert equation ($A = \varepsilon cl$), where ε is the molar extinction absorption coefficient (also known as molar absorptivity or sometimes spectral absorption coefficient) for the absorbing material in molars $dm^3\ mol^{-1}\ cm^{-1}$ (this is wavelength dependent and usually measurements are carried out at the wavelength of maximum absorption), c is the concentration of the absorbing material in moles and l is the optical path length of the absorbing material in cm.

As with IR, this technique is not capable of separating components, thus if two compounds are present that both absorb at the same frequency then they both contribute to the overall absorbance of the sample being analysed. However, it is possible if at least one of the components has a unique absorbance band to carry out at least partial quantitative analysis. Again advances in software, including data manipulation, such as taking derivatives, for example looking at the rate of change of absorbance with respect to wavelength, can sometimes help to resolve the data for simple mixtures of dyes.

In cases where confirmation of identity is required, the UV and/or visible spectrum of a colorant may supplement the IR spectrum or any other means of identification. Note that a UV/visible absorbance spectrum is unlikely to be sufficiently unique to be conclusive on its own.

UV/visible systems often make very good detectors to attach to other instruments, especially liquid chromatography and capillary electrophoresis, that separate the components prior to their spectroscopic detection. Fixed wavelength detectors, multiple wavelength detectors, variable wavelength detectors and diode array detectors exist to monitor UV/visible light absorbance of a mobile phase (carrying analytes) after it has passed through a separation column or capillary (as in high-performance liquid chromatography and capillary electrophoresis). Fluorescence detectors are also sometimes utilised.

UV/visible spectrophotometry has been and continues to be important in the characterisation of dyes (with literally thousands of papers on dyes having some mention of this technique) and, additionally, can be used to monitor the decolorisation of solutions. One interesting collection of papers that helps to highlight the potential usefulness of UV/visible spectrophotometry is that produced by Oakes and coworkers.[32–35]

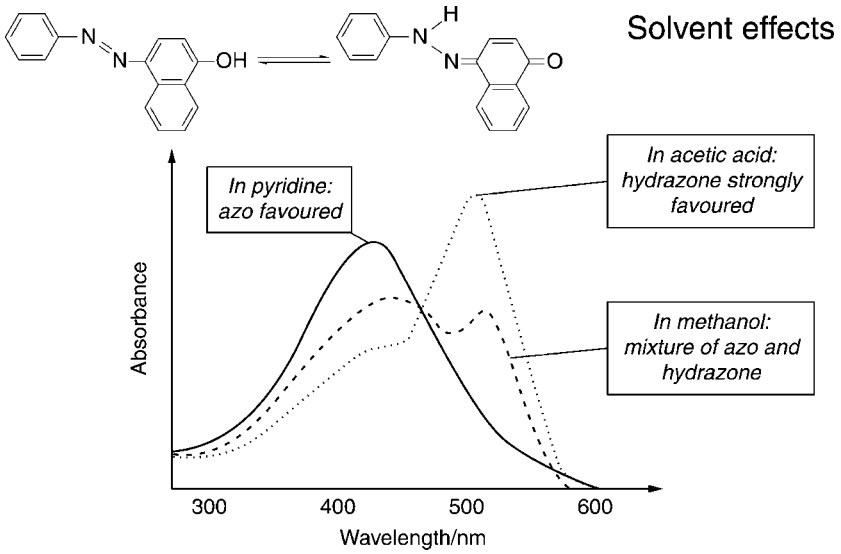
However, to obtain more information (especially for mixtures or unknowns) chromatographic techniques are frequently used, as discussed later.

The pH sensitivity of some dyes (some are used as pH indicators) are well known, others less so. Solvatochromism in colorants can also be important and similarly be investigated by UV/visible spectrophotometry (an example of this is shown in Fig. 10.5).

The use of online colour measurement for process control purposes has been reviewed by Gilchrist[36] and Ferus Comelo.[37]

10.4.5 Mass spectrometry

Mass spectrometry (MS) has become one of the most versatile analytical techniques since a variety of interfaces can be used in order to analyse a wide range of sample types in different physical states (both volatile and non-volatile) from different sources. The fact that MS can be hyphenated to several other techniques in order to, for example, resolve components in mixtures prior to the MS analysis, makes it an extremely powerful technique. The good sensitivity and high specificity



10.5 Solvent effects on the absorbance spectrum of an azo-hydrazone dye.

of MS means that it would be the method of choice (subject to available funds) in the analysis of a wide range of samples, including many environmental samples. MS has been widely used in the analysis of dyes for many years. Applications of liquid chromatography-mass spectrometry (LC-MS) in environmental chemistry is the subject of a book edited by Barceló, which includes sections on the analysis of dyes by LC-MS.[38]

10.5 Atomic spectroscopy (and elemental analysis)

Atomic spectroscopy is employed for the qualitative and quantitative determination of around 70 elements; primarily for the analysis of a wide range of 'metals' (often for trace analysis). Atomic spectroscopy can provide information regarding the identity and concentration of atoms in a sample irrespective of how these atoms are combined. In contrast, molecular spectroscopy gives qualitative and quantitative information about the molecules (or particular functional groups present in molecules) in a sample.

Sensitivities of atomic methods lie typically in the parts per million (mg dm^{-3}) to parts per billion (μg dm^{-3} or μg kg^{-1}) range, although in some cases in the parts per trillion (ng dm^{-3}) range. (You may wish to think about the implications of this !!!). Additional virtues associated with these methods include speed, convenience, unusually high selectivity and moderate instrument costs (although not for an inductively coupled plasma–mass spectrometry system!).

The first key step in all atomic spectroscopic techniques is 'atomisation', a

process in which a sample is volatilised and decomposed in such a way as to produce an atomic gas. This is by far the most important step in atomic spectroscopy. As atomic spectroscopy relies on the analyte being atomic, the efficiency and reproducibility of atomisation will largely determine the entire method's overall sensitivity, precision and accuracy.

Atomic emission spectra are produced when an atom or ion excited by the absorption of energy from a hot source relaxes to its ground state by giving off a photon of radiation (with a characteristic wavelength). In contrast, atomic absorption takes place when a gaseous atom or ion absorbs a photon of radiation (with a characteristic wavelength) from an external source and is thus excited.

Atomic absorption spectroscopy (AAS) is probably still the most widely employed of all the atomic methods because of its simplicity, effectiveness and relatively low cost. A 'line source' of radiation is required for AAS (they do not employ a continuous source of radiation) hence a complete spectrum is not obtained. The sources (which are changed depending on the element of interest) emit certain lines of radiation that have the same wavelength as that of the absorption peak of the analyte of interest.

Sample preparation is again a key step in the analysis. The sample to be analysed is usually in solution in order to be efficiently introduced and atomised in the flame or plasma. For many solid samples, such as dyed/printed textiles, this will involve digestion in strong acid followed by ashing in a furnace (to break down organics and drive off carbon and hydrogen). After ashing the sample is taken up in some acid and diluted to volume prior to atomic analysis.

Atomisation in plasmas

Commercial plasma atomisers became available in the mid 1970s and offer several advantages over flame atomisers. A plasma is a conducting gaseous mixture containing a significant concentration of cations and electrons.

In the argon plasma employed for emission analyses, argon ions and electrons are the principal conducting species, although cations from the sample also contribute. Argon ions, once formed in the plasma, are capable of absorbing sufficient power from an external source to maintain the temperature at a level at which further ionisation sustains the plasma indefinitely; temperatures as great as 10 000 K are encountered. Of the power sources investigated, the radiofrequency, or inductively coupled plasma (ICP), source was found to give the greatest advantage in terms of sensitivity and freedom from interference. The excellence of these results stems from the high stability, low noise, low background and freedom from interferences of the sources when operated under appropriate experimental conditions.

Generally, the detection limits with the ICP source are comparable to or better than those of other atomic spectral procedures. Additionally, ICP allows you to scan for multiple analytes simultaneously, unlike AAS, for instance, which

traditionally can only test for one element at a time (although modern instruments and lamps are now available that have a limited capability for multi-element analysis, for certain popular elements). The biggest disadvantage of ICP is that the instrumentation is expensive to purchase.

Reasons for metal ions/elements to be present in colorants include: they are an integral part of the colorant, such as in an inorganic pigment or a metal–dye complex; they are an impurity, possibly from a raw material/intermediate, a reagent/catalyst used in the synthesis or a species present in the water/media used during synthesis/isolation.

Readers should note that other analytical techniques also exist for investigating the elemental make up of samples, such as CHN analysers (especially for compositional analysis of pure organic chemicals) and X-ray fluorescence (XRF) instruments, and techniques such as X-ray photoelectron (XPS) spectroscopy are available for surface-specific analysis, but expensive.

10.6 Separation science

Most real samples that are analysed are, unless they have been deliberately purified (and even then they may still be), actually made up from a number of different chemicals; this is certainly true for most colorants. As has already been discussed when considering molecular spectroscopy techniques, analysis of mixtures can lead to complex, incomplete or even unresolvable data. The answer/solution to problems of this type normally involves separation science, where there is selective or differential interaction/behaviour of the different components in the separation system. The principle separation sciences are chromatography and electrophoresis.

10.6.1 Chromatography

Chromatography is generally considered to be a technique for separating (and identifying) organic molecules, especially those of low to medium molecular masses (typically up to about 1000 atomic mass units, amu), although chromatography is much broader than this (with variations, mostly in the technology of the stationary/fixed phases, for small inorganic ions and large macromolecules). The separation of colorants has been a very popular application area for chromatography techniques over the 100 years since its initial development. In fact its first reported use was the separation of plant colorants, which lead to its name ‘chromatography’ – colour writing. Chromatographic techniques are many and may be employed on a micro scale for quantitative and qualitative analysis or may be adapted to a macro scale for preparative work.

Dyes have been traditionally analysed by thin-layer chromatography (TLC) and then more recently by high-performance liquid chromatography (HPLC). GC is not normally suitable for dyes since dyes are generally non-volatile ionic or strongly polar compounds. The most popular form of dye analysis by HPLC

usually employs a reversed phase column and an ion-pairing agent to complex with the charged sites on the dye molecule. Advances in chromatography for dyestuffs have been reviewed by Evans and Truslove.[39]

Thin-layer chromatography (TLC)

Thin-layer chromatography is generally considered to be a low-tech approach that is cheap and cheerful, which is especially useful for quick checks on samples in almost any location (from the factory floor to the research laboratory to out in a field!). Whilst it is normally used in this way, it can also be linked with automatic densiometers and spectrophotometers to form quite a sophisticated analysis system capable of generating quantitative as well as qualitative results.

Examples of applications include:

- monitoring the synthesis of a colorant (following the consumption of raw materials – often using a UV lamp to check for their presence, the formation of the required product(s) – a coloured spot or spots, the formation of side products (again may be coloured) and the formation of impurities);
- checking the organic purity of a colorant (note, inorganic species, such as salts will not show up), although care should be taken as an organic impurity may not be 'seen' – either masked by an analyte spot or, if it does not absorb visible light (and it is not checked for with a UV lamp), it may just be 'missed';
- checking an environmental sample (possibly from a watercourse or an extracted sample, e.g. extracted from soil) for the presence of colorants;
- following the decolorisation/breakdown of colorants (e.g. via a biochemical or chemical reaction).

Often analysis by TLC will be followed up by further analysis using liquid chromatography (or capillary electrophoresis), especially if more quantitative analysis is required.

Gas chromatography (GC)

In gas chromatography (GC) the mobile phase is a gas (e.g. nitrogen, N_2) and moves rapidly compared to the movement of the separated substances. Thus, R_f values (retardation factor, i.e. the distance travelled by an analyte divided by the distance travelled by the mobile phase (these values are routinely used in TLC)) would be very small and hard to determine. Retention times are therefore employed in preference to R_f values. The retention time of a compound (also used in liquid chromatography) may be described simply as the length of time it takes a compound to be detected following injection. This quantity has units of time, that is, seconds or minutes.

GC is sometimes referred to as gas liquid chromatography (GLC) since the stationary phase in modern capillary columns behaves (although it is normally

covalently bonded to sites on the internal wall of the capillary) as if it is a liquid and the analytes partition into this phase. GC column packings and liquid phases are available in considerable diversity to achieve sharp separation of a variety of compounds. The oven may be operated isothermally or temperature programmed to as high as about 350 ºC depending upon the particular packings and liquid phases employed, this allows separation of compounds of widely different volatility. To improve resolution and reduce tailing, some polar compounds, such as acids, alcohols and amines, may first be converted to a derivative. For example, conversion of acids to methyl esters is common.

Most colorants, because of their ionic (most dyes) or particulate (pigments) nature with strong intermolecular forces and low volatility cannot be analysed by GC, however, lower molecular weight species, such as certain starting materials, impurities, additives and breakdown products from colorants can be analysed. These include aromatic amines such as those in the German MAK III list, which were discussed earlier in this chapter in Section 10.2.2.

A frequent use of GC with polymers is in the quantitative determination of residual monomer and solvent content, even at sub parts per million levels. This is especially important in food contact applications (e.g. printed packaging materials) where taint and odour issues are important. There are a range of sample preparation and injection techniques to deal with a vast range of samples including, for example, 'headspace' sampling where a solid can be incubated for a period of time and then the vapours from above the solid are transferred into the GC capillary.

There are a variety of detectors for GC systems, however, mass spectrometry (MS) is generally accepted as the best overall. GC-MS is a very powerful and popular technique and therefore has a wide range of different application areas. The major reasons for using GC-MS are:

- identification of unknown analytes;
- target analysis – looking for specific compounds (usually quantitative analysis, requiring suitable calibration);
- post chromatography separation (it is possible to 'separate' and resolve co-eluting analytes in the MS);
- matrix elimination (when MS is needed).

Pyrolysis

Identification of non-volatile compounds by GLC can be carried out using pyrolysis to decompose the polymer into volatile products prior to analysis by GLC. Pyrolysis chromatography constitutes an important section of gas chromatography practice. It is of particular use in characterising compounds that have distinctive thermal degradation products.

The pyrolytic chromatogram is sometimes called the pyrogam. In most cases it is difficult (especially without MS detection) to identify accurately the decomposition products because of their large number. Such pyrograms are primarily suited

to comparisons as 'fingerprints' or for the detection of the presence of certain components or contaminants. The pyrolytic chromatogram is analogous to an IR spectrum as a 'fingerprint' of the compound. Comparison of the chromatogram of an unknown with those of reference standards may permit a positive identification.

Inverse gas chromatography (IGC)

An alternative way of using gas chromatography is exploited in the technique of 'inverse gas chromatography'. IGC allows for the investigation of a stationary phase (e.g. polymer or pigment) with 'unknown' properties to be studied by passing compounds (probes), whose properties are known, through the packed column/capillary and studying the interactions that occur; this type of information can be particularly useful when considering compatibility and behaviour of components (including colorants) in formulations (such as paints and inks).

Liquid chromatography

For charged species, such as most dyes, reversed phase (RP) ion-pair chromatography is employed.

Ion-pair (or paired ion) chromatography is a form of reversed-phase partition chromatography that is employed for the separation and determination of organic ionic species. The mobile phase in ion-pair chromatography consists of an ion pairing agent, containing a counter ion of opposite charge to the analyte(s) of interest, in an aqueous buffer based on an organic solvent such as methanol or acetonitrile. The combination of the counter ion with the analyte ion results in an ion pair. The actual ion pair is more hydrophobic than the parent dye ion (and may be a neutral species) and will demonstrate some degree of retention on a reversed-phase packing material (usually a C18 or a C8 column). For anionic dyes quaternary ammonium species are used as the counter ion, with probably the most common ion-pairing agent being tetrabutylammonium bromide [$(C_4H_9)_4N^+ Br^-$].

10.6.2 Capillary electrophoresis (CE)

Capillary electrophoresis (CE) is a relatively new separation technique and often comparisons are made to the better established technique of high-performance liquid chromatography (HPLC), since they are generally capable of analysing a similar wide range of chemicals. It should be emphasised, however, that the separation principles are quite different. Conventionally in CE, species are separated based on their electrophoretic mobilities (determined mainly by their mass to charge ratios), in an aqueous electrolytic buffer media inside a fused silica capillary. The two ends of the capillary are immersed in separate reservoirs of the buffer and a high voltage is applied across the capillary to induce a bulk (electro-osmotic) flow of buffer through the capillary. Note that the detector (usually a

Table 10.5 Factors affecting capillary electrophoresis separations

Factor	Variables to be considered
Analytes	Nature, state, concentration, solubility and solvent
Buffer	Type, additives, concentration, pH
Capillary	Length, diameter, surface properties, maintenance
Detection	Type, sensitivity
Separation Parameters	Current strength, temperature, separation time

UV/visible detector) is on-line (actually measuring the analytes whilst they are still in the analytical capillary).

The technique is therefore particularly suited to water-soluble species which possess a charge, which includes most dyes. Variations from the main technique (often referred to as capillary zone electrophoresis, CZE) do exist and these include the use of surfactants in the buffer to create micelles (often thought of as creating a pseudo-stationary phase inside the capillary) which then improve the separation of neutral species (and frequently charged molecules) owing to hydrophobic interactions. The term micellar electrokinetic chromatography (MECC or MEKC) is often used for CE separations carried out with the aid of a micellar buffer solution.

Factors affecting capillary electrophoresis analysis

There are many factors that can affect the results that are obtained from the analysis of samples by CE and the major ones are listed in Table 10.5.

One of the most important variables, as with many techniques, is the 'state' of the sample to be analysed. Questions which should be considered prior to CE analysis include: What is the sample dissolved in? What are the known or likely impurities/additives present? How might these 'impurities' affect electrophoresis results? Are sample modifications possible under the separation conditions employed (e.g. pH or temperature-sensitive compounds)? Could aggregation or precipitation be a problem? What is the concentration of the sample – does it need to be diluted or concentrated? Finally it is important to consider any known properties of the samples to be analysed, for example charge, spectral absorption and adsorption characteristics, as these will aid in the setting of some of the other variables for the analysis.

Typical applications in CE

Commercial CE instruments only became readily available in the late 1980s, however, a vast array of information about different applications has been published. As well as the dyes themselves, CE can be used, for example, for the analysis of low molecular weight/simple ions (such as small anions, e.g. chloride, bromide, nitrate, chlorite, acetate, phthalate or small cations, e.g. alkali + alkali earth metals, ammonium, transition metals) or for aromatic amines.

The high resolving power of CE techniques has been recognised and investigated by many scientists. The use of CE for the analysis of environmental samples has been extensive. Good sampling, handling and preparation procedures are usually crucial in environmental analysis. A comprehensive review of different handling and preparation techniques for environmental samples analysed by CE has been presented by Brumley;[40] consideration was given to samples contained in both aqueous and solid matrices.

CE is ideally suited to the analysis of most dyes. Relatively simple aqueous buffers such as phosphate, borate and citrate, depending on the nature of the dyes, have been found to be suitable for a wide range of dyes.[41–44] The use of micellar buffer systems have proved beneficial for the analysis of some dyes, their intermediates, precursors and impurities.[45–48] These analyses have been either with or without an organic modifier depending on factors such as the nature of the analytes and the matrix they are in, since commercial dyes exist in formulations which, in addition to possibly being a blend of more than one dye, may contain a wide range of diverse components. The fact that dyes absorb light in the visible region, as well as the UV region, of the electromagnetic spectrum means that a greater degree of selectivity can be obtained by using detectors which are capable of monitoring absorbance of light in the visible region.

When analysing reactive dyes care needs to be taken in the interpretation of the results. Certain ranges of reactive dyes may be supplied in the form of a precursor of the actual reactive dye. Reactive dyes can undergo hydrolysis in aqueous environments (especially if alkaline). In recent years there has been an increase in the number of bifunctional reactive dyes. For all of the reasons mentioned, a multitude of peaks may be detected from the different derivatives of dyes such as Remazol Black B (C.I. Reactive Black 5).[48] The full chemistry of reactive dyes has not always been appreciated by workers analysing them leading to misinterpretations, for example in the work of Oxspring *et al*.[44]

10.7 Summary of instrumental analysis

There are many reasons for the analysis of colorants, for example, from trying to identify the structure of a new compound, to checking the purity of a known colorant, to checking the concentration of known/target impurities associated with a particular compound, to monitoring processes involving colorants (synthesis/coloration/decolorisation), to trying to identify (and often to also quantitate) unknown colorants (possibly at trace levels) in 'complex' samples. Legislation has become a bigger driving force behind quite a lot of this legislation and, as developments (especially lower limits of detection) in analytical techniques have been made, difficult questions surrounding the safety (and the safe level, since specifying 'undetectable' is not sensible/realistic) of chemicals including colorants need to be carefully considered. Analysis costs for the coloration industries are significant. There are of course companies making money out of all this

analysis, thus appreciation of the possibilities, pitfalls and implications of analysis (and also not doing analysis) is important for companies.

There are a wide range of techniques and a vast array of methods associated with these techniques. This chapter has covered a great number of techniques, but there are gaps. Hopefully, after reading this chapter the reader will have a better appreciation of colorants, issues associated with the analysis of colorants and some of the techniques and methods used for analysing colorants.

Active areas of research and development in terms of analysis include miniaturisation of analytical instrumentation with the idea of 'Lab on a chip'; this could possibly lead to mass production and thus cheaper instruments, so much so that for some techniques they become disposable (this should also make analysis much quicker). The hyphenation of techniques continues to be a very active area owing to the potential benefits that can accrue from linking techniques together, work on the interfaces usually being key. Work on sensors is ongoing for both specific chemicals and groups of related chemicals. There is also a lot of ongoing effort into developing process analysis (with the monitoring and auto-feedback, leading to greater control and optimisation) with spectroscopic techniques probably receiving the most attention. Many of the developments will not necessarily be targeted at colorants first, but opportunities will arise from developments/applications demonstrated with other chemicals, as has happened previously.

10.8 Colorant analysis without using instruments

The analysis of dyes and pigments for textile applications is really a very broad subject because it usually involves many different types of analyses and the use of many sophisticated instruments. It is difficult to provide a detailed coverage in a short section here. Therefore, only some basic and simple analyses are introduced. Readers who want to know more can access the books published elsewhere.[49,50] Analysis of colorants can also mean many different things. Identification of colorants on textile materials is an important aspect of forensic analysis and also very useful for textile dyers. Characterisation of colorants is critical for colour chemists to know what chemical structures the colorant has. Determination of dye classes has its practical significance in making up dyeing and printing recipes as well as in the analysis of historical textiles.

10.8.1 Dye purification and detection of ionic type

When commercial colorants reach the users, usually they are in the form of liquid or solid. The liquid form can be in dispersion, emulsion or high concentration solution. The solid form can be in granule or powder. The effective assay of solid dyes is usually between 85 and 95% with the exception of indigo which can have a purity level as high as 99.5%. For the analysis of the colorants in their original package, dyeing using standard fabrics under standard conditions developed by

individual laboratories can be used as a quality check tool. This quality check is a necessity for quality assurance purposes. It is the easiest and most convenient way to determine the quality of the colorants. Before the colorant is used in mass production, a lab dip can always give an indication of whether or not the colorant is up to the standard in terms of both the colour strength and the dyeing performance, including the fastness properties of the dyeing which are often the determining factors for a dyeing operation. Of course, a simple spectrophotometric measurement of a colorant sample solution can indicate the colorant strength if the standard reading of the same colorant is available.

If an unknown colorant needs to be analysed, an imporatant step is usually the removal of salt from the dye sample. The salt removal method quoted by Mehta *et al*.[51] can be used for dyes containing the sulphonate group. The dye sample is first dissolved in a minimum volume of cold dimethylformamide (DMF) and then filtered through a sintered glass funnel. Acetone is then added to the stirred filtrate and the dye recrystallised. The solution should be cooled in a fridge to aid the recrystallisation over night. If necessary, the procedure should be repeated until the UV/vis absorbance of an aqueous solution of the dye sample is constant. The advantages of this method are that it can avoid the dye hydrolysis and eliminate the possibility of water of crystallisation in the samples, which would interfere with the elemental analysis results of dye analysis. Another method for salt removal of sulphonated dyes involves the use of sodium thiocyanate.[52] The dye sample is first dissolved in a minimum amount of distilled water. Solid sodium thiocyanate is added into the dye solution to precipitate the dye. The precipitated solid is washed with acetone to remove the sodium thiocyanate until the spend acetone shows no trace of thiocyanate peak in the vicinity of 2080 cm^{-1} in its FT-IR spectrum. One of the advantages of using this method is that water soluble dyes are insoluble in acetone, so no dye is lost in the washing step. The dye obtained in this way is readily redissolvable in water.

It was also reported that some alcohol-soluble dyes like C.I. Direct Yellow 12 can be purified using a mixture of toluene and absolute alcohol.[53] Some water-soluble and alcohol-insoluble dyes can be purified from a 60:40 ethanol:water mixture.[54] Precipitation with sodium acetate repeatedly can give a highly purified dye.[55] This method involves as many as five times of precipitation, filtration and redissolving. The final precipitate will be washed with boiling ethanol until no acetate is detected in the spend alcohol by the cacodyl test.[56]

The ionic type of a dye sample can be determined by using ionic surfactants. The anionic dyes can be precipitated by cationic surfactants and vice versa. If a colorant sample would not be affected by either cationic or anionic type of surfactants, it could be a disperse dye with non-ionic dispersants. The surfactant test should be carried out at room temperature and a surfactant solution is added into the dye solution dropwise with the help of a magnetic stirrer. The mixture should then be allowed to stand for 30–60 min for precipitation to develop. Precipitation is the positive indication of the opposite type of ion for dyes in relation to the ionic

surfactant used. With a carefully designed analytical procedure using standardised surfactant solutions, the colorant concentration in the sample can be determined. An experienced textile chemist should also be able to use other types of ionic compounds to do similar analysis, both quantitatively and qualitatively.

10.8.2 Determination of dye purity level

The methods presented here are classical analyses. They are based upon either a redox reaction or an acid–base neutralisation reaction. Since there are a variety of chemical constituents, these methods have their limitations.

Reduction with titanous chloride[57]

This method is suitable for almost all azo dyes and some other water-soluble dyes. The titration should be conducted in a well-ventilated fume hood. A dye sample is dissolved in a hydrochloric acid solution. An excess amount of titanous chloride solution is added into the boiling acidic dye solution under the protection of a CO_2 atmosphere. A back titration is then conducted using a standardised ferrous ammonium sulphate solution with ammonium thiocyanate as the indicator. The titanous chloride solution should be freshly standardised.

Oxidation with potassium dichromate[58]

This method is suitable for nitrogen-containing dyes. The analysis is based on the measurement of nitrogen gas released from the strong oxidation reaction. A dye sample solution is boiled together with dichromate solution in the presence of sulphuric acid. The nitrogen gas generated is collected in a stream of CO_2 and measured. Before the start of the reaction, the entire reaction assembly is purged and filled with CO_2. Some nitrogen-containing impurities can lead to errors.

Titration of acid dyes with basic compounds[54]

A solution of a basic compound, such as Fixanol,[59] which is essentially cetyl pyridinium bromide and cetyl trimethylammonium bromide, is added to the acid dye sample solution to precipitate the acid dye. The end point of the titration is reached when the colour of a drop of the titrated solution on filter paper is different from that of the precipitate. The drawback of this method is that the end point determination is difficult, which could lead to titration errors. A few repeated titrations may reduce the error level.

10.8.3 Identification of dyes on fibres

There are a few sets of identification systems available. A scheme developed by

Green is the first known useful system for identifying the classes of dyes on wool and cellulosic fibres.[60] Clayton updated the Green system with more effective reagents and covering more dyes.[61] Hurwitz, Salvin and McConnell presented a system to identify dyes on cellulosic fibres, animal fibres and synthetic fibres in an AATCC publication.[62]

According to the AATCC system,[62] dyes on cellulosic fibres can be divided into four groups. Group 1 is basically for direct dyes. A dyed sample is boiled with an ammonia solution to extract the dye from the dyed sample. The dyed sample is then removed from the coloured extraction solution and a white piece of cotton fabric and a small amount of salt are put into the same solution for redyeing at the boil for about 1 min. A direct dye is evidenced by the similar shade and depth of the redyed cotton fabric in comparison to the original dyed sample. Group 2 dyes can go through first reduction, then oxidation reactions and can revert to their original colours. They are usually vat dyes and sulphur dyes. The sample usually should be tested for Group 1 before testing for Group 2. The reduction reaction of the dyed sample is carried out using an alkaline sodium hydrosulphite solution at the boil for 2–5 s. The discoloured dyed sample is removed from the reduction bath, dried on filter paper and left to oxidise in air. If the original colour is recovered in about 5 min, it is the positive indication of sulphur or vat dye on the original dyed sample. For sulphur dyes, the confirmative indication is a reduction with sodium sulphide in the presence of sodium carbonate and redyeing of a white piece cotton fabric to a same but lighter shade with the same reduction bath plus some salts. Group 3 dyes are those dyes that can be reduced by sodium hydrosulphite but cannot be oxidised back. They are direct dyes after-treated with metals and formaldehyde, naphthols and insoluble azo dyes, and diazotised and developed dyes. These dyes are all damaged by the reduction reaction at prolonged boiling. Group 4 is for those colorants that cannot be identified by the tests listed in the first three groups. They are very likely to be pigments or maybe reactive dyes. Pigment cannot penetrate the fibre. Therefore a microscopic examination of the pigment coloured fibre cross-section will display a circular coverage of the fibre surface. A DMF extraction can also be used to distinguish pigments from reactive dyes. A 100% DMF treatment at the boil can extract pigments but not reactive dyes from coloured samples. DMF can also be used as a 1:1 DMF:water solution for the identification of other dye classes. The possible results are shown in Table 10.6.[62]

If a fibre sample can be dissolved in 5 % NaOH at the boil, it is a protein fibre, commonly wool or silk. Protein fibres can be dyed most often by acid dyes, metallised acid dyes and chrome dyes. According to the AATCC system, acid dyes on protein fibres can be identified as follows. A coloured sample is boiled in an ammonia solution for 1–2 min. After removing the coloured sample, the ammonia solution is slightly over-neutralised with sulphuric acid. A small piece of white wool sample is then redyed with the ammonia solution at the boil for 1–2 min. A positive indication of acid dyes on the original sample is evidenced by the colour of the redyed wool sample. If chrome dyes are on the dyed sample, no redyeing can

Table 10.6 DMF extraction test of dyes at boiling

1:1 DMF:water	100% DMF
Extraction coloured by:	Extraction coloured by:
All direct	Vats
Diazotized and developed	Leuco vats
Some basic	Naphthols
Some mordant	Sulphurs
	Pigments
Extraction not coloured by:	Some basic
Fibre reactive	Some mordants
Leuco vat	
Naphthols	Extraction not coloured by:
Pigments	Fibre reactive
Some basic	
Some mordants	

occur on the white wool fabric. Metallised dyes are characterised by the presence of chromium, cobalt or manganese. An ash test should be able to distinguish between them. The dyed sample is fused with five times its amount of a mixture of equal parts of sodium carbonate and sodium nitrate in a crucible. A royal blue colour of the cooled melt indicates the presence of cobalt; yellow, chromium; and blue-green, manganese.

The AATCC system is a very detailed dye class identification system. The brief description of some basic tests in this chapter is only for the purpose of introduction. Readers are recommended to obtain the book *Analytical Methods for a Textile Laboratory* by the AATCC in order to comprehend the complexity of the dye analysis. Of course the other books and journal articles listed in the references are also excellent information sources. Whenever possible, reading these materials would definitely help develop a better understanding of colour chemistry, which will ultimately ensure that the dye analysis is performed more effectively and efficiently.

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