Grzegorz Zadora | Agnieszka Martyna | Daniel Ramos | Colin Aitken

Statistical Analysis in Forensic Science

Evidential Value of Multivariate Physicochemical Data

$$LR = \frac{f(\bar{\mathbf{y}}_1, \bar{\mathbf{y}}_2 \mid \mathbf{U}, \mathbf{C}, \mu, H_1)}{f(\bar{\mathbf{y}}_1, \bar{\mathbf{y}}_2 \mid \mathbf{U}, \mathbf{C}, \mu, H_2)}$$

$$\mathbf{X} = \begin{bmatrix} x_{11} & \cdots & x_{1j} \\ \vdots & \ddots & \vdots \\ x_{i1} & \cdots & x_{ij} \end{bmatrix}$$

$$\mathbf{Y} = \begin{bmatrix} y_{11} & \cdots & y_{1j} \\ \vdots & \ddots & \vdots \\ y_{i1} & \cdots & y_{ij} \end{bmatrix}$$

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To our families

Contents

Preface

An increase in the danger from new forms of crime and the need by those who administer justice for higher standards of scientific work require the development of new methods for measuring the evidential value of physicochemical data obtained during the analysis of various kinds of trace evidence.

The physicochemical analysis of various types of evidence by the application of various analytical methods (Chapter 1) returns numerous types of information including multivariate quantitative data (Chapter 3, Appendix B), for example, concentrations of elements or the refractive index of a glass fragment. The role of the forensic expert is to evaluate such physicochemical data (evidence, E) in the context of two competing propositions H_1 and H_2 (Chapter 2, Appendix A). The propositions H_1 and H_2 may be put forward by the police, prosecutors, defenders or the courts and they concern:

- *comparison problems* (Chapter 4), for example where H_1 states that the glass samples being compared originate from the same object, and H_2 that the glass samples being compared originate from different objects;

- *classification problems* (Chapter 5), for example where H_1 states that the glass sample which has been analysed originates from a car or building window, and H_2 states that the glass sample analysed originates from a container glass.

Bayesian models have been proposed for the evaluation of the evidence in such contexts. Statistical analysis is used to evaluate the evidence. The value of the evidence is determined by the likelihood ratio (LR). This is the ratio of the probability of the evidence if H_1 is true, $P(E \mid H_1)$, to the probability of the evidence if H_2 is true, $P(E \mid H_2)$. For evidence in the form of continuous data these probabilities are replaced with probability density functions, $f(E \mid H_1)$ and $f(E \mid H_2)$.

The LR approach (Chapter 2, Appendix A) has become increasingly popular for evidence evaluation in forensic sciences. For physicochemical data, the approach enables an objective evaluation of the physicochemical information about the analysed object(s) obtained from an analytical run, and about the rarity of the determined physicochemical features for recovered and/or control samples within a relevant population (Chapters 2, 4 and 5). The most common application of the LR approach in forensic science is in DNA profiling. The LR approach has also been applied to other evidence categories including earprints, fingerprints, firearms and toolmarks, hair, documents, envelopes and handwriting, and speaker recognition. In recent years, much has also been published on LR approaches for multivariate data. Some of these ideas (including examples from practice in forensic science) are discussed in this book.

The performance of each statistical approach should be subjected to critical analysis, not only in the form of error rates but also through the use of other formal frameworks which provide a measure of the quality of a method for the evaluation of evidence based on a likelihood ratio. There is a need not only for the measurement of the discriminating power of the LR models as represented by false positive and false negative rates, but for the information that the LR provides to the inference process in evidence evaluation, where the important concept of calibration plays a significant role. One of the objectives of this book is to consider the problem of the assessment of the performance of LR-based evidence evaluation methods. Several methods found in the literature are extensively described and compared, such as Tippett plots, detection error trade-off plots (DET) and empirical cross-entropy (ECE) plots (Chapter 6, Appendix C).

One reason for the slow implementation of these LR models is that there is a lack of commercial software to enable the calculation of the LR relatively easily by those without experience in programming (like most forensic experts). Therefore, in order to use these methods case-specific routines have to be written using an appropriate software package, such as the **R** software (www.r-project.org). Based on information gathered during workshops on statistics for forensic scientists (e.g. the "FORSTAT – Forensic Statistics" project under the auspices of the European Network of Forensic Sciences Institutes), the present authors believe that there is a need for a book that provides descriptions of the models in more detail than in published papers, as well as of the software routines, together with practical examples. Therefore, the aims of this book are to present and discuss recent LR approaches and to provide suitable software toolboxes with annotation and examples to illustrate the use of the approaches in practice. The routines included in the book are available from the website www.wiley.com/go/physicochemical. These include routines in **R** (Appendix D), precomputed Bayesian networks for **Hugin Researcher**TM (Appendix E), and the **calcuLatoR** software (Appendix F) for the computation of likelihood ratios for univariate data. Manuals (Appendices D–F) including examples and recommendations of the use of all of these assessment methods in practice are included, as well as software and practical examples to enable forensic experts to begin to work with them immediately (Chapters 3–6).

Note also that the LR approaches presented in the book can be used whenever evidence is to be evaluated under the circumstances of two propositions. Therefore, the models described in the book can also be applied in other areas of analytical chemistry. Special emphasis is placed on the solution of problems where a decision made on the basis of results of statistical analyses of physicochemical data could have serious legal or economical consequences; thus, for example, one of these other areas of analytical chemistry could be that of food authenticity analysis.

Many people have helped in many ways in the preparation of this book, too many to enable us to acknowledge them all individually. However, we wish to acknowledge in particular Rafal Borusiewicz, Jakub M. Milczarek, David Lucy, Tereza Neocleous, Beata M. Trzcinska, and Janina Zieba-Palus for many helpful discussions and a great deal of collaborative work, from which we have been able to take much inspiration for the content of the book.

We also wish to thank Christopher J. Rogers. He checked the examples from the perspective of a beginner in the determination of the evidential value of physicochemical data. His suggestions helped to improve the quality of the practical examples contained herein.

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1

Physicochemical data obtained in forensic science laboratories

1.1 Introduction

Various materials can be subjected to physicochemical examination by forensic experts. Such materials include illegal substances, blood and other body fluids, and transfer evidence (e.g. small fragments of glass, paint, fibres, plastics, organic and inorganic gunshot residues, fire debris). The size of samples subjected to analysis is very small, for example, fragments of glass with a linear dimension below 0.5 mm. Therefore, the analysis of morphological features, such as thickness and colour, is of no value for solving a comparison (Chapter 4) or classification problem (Chapter 5). Thus, it is necessary to employ the physicochemical features of the analysed fragments. When choosing an analytical method for analysis of microtraces for forensic purposes an expert should take into account not only the fact that the amount of material is very small but also that the method chosen should be non-destructive, leaving the material available for reuse. Examinations performed by the application of various analytical methods return several kinds of information including:

- qualitative data, for example, information on compounds detected in fire debris samples based on a chromatogram, information obtained from the spectrum of an unknown sample, and morphological information such as the number and thicknesses of layers in a cross-section of car paints;

- quantitative data, for example, the concentration of elements or value of the refractive index in a glass fragment, peak areas of a drug profile chromatogram or gasoline detected in fire debris, and the concentration of ethanol in blood samples.

In general, the fact finders (i.e. judges, prosecutors, policemen) are not interested in the physicochemical composition of a particular material (e.g. the elemental composition

of glass) except in situations where such information could have a direct influence on the legal situation of a suspect (e.g. information on the level of ethyl alcohol in a blood sample). Questions raised by the police, prosecutors and the courts relate to the association between two or more items (which is known in the forensic sphere as a *comparison problem*; Chapter 4) and/or identification and classification of objects into certain categories (known in the forensic sphere as a *classification problem*; Chapter 5). These problems can be solved by the application of various statistical methods.

There are two main roles of statistics in forensic science. The first is during the investigation stage of a crime before a suspect has been identified, where statistics can be used to assist in the investigation (Aitken 2006a). The second is during the trial stage (Aitken 2006b), where statistics can be used to assist in the evaluation of the evidence. This last role of statistics is described in detail in this book.

When the evaluation of evidence is based on analytical data obtained from physicochemical analysis, careful attention to the following considerations is required:

- possible sources of uncertainty (sources of error), which should at least include variations in the measurements of characteristics within the recovered and/or control items, and variations in the measurements of characteristics between various objects in the relevant population (e.g. the population of glass objects);

- information about the rarity of the determined physicochemical characteristics (e.g. elemental and/or chemical composition of compared samples) for recovered and/or control samples in the relevant population;

- the level of association (correlation) between different characteristics when more than one characteristic has been measured;

- in the case of the comparison problem, the similarity of the recovered material and the control sample.

In this book it is advocated that the best way to include all these factors in the evidence evaluation process is by the application of likelihood ratio (LR) approach (Chapter 2).

It was mentioned that results of physicochemical analysis of various types of forensic evidence can be enhanced using statistical methods. Nevertheless such methods should always be treated as a supportive tool and any results should be subjected to critical analysis. In other words, statistical methods do not deliver the absolute truth as the possibility of obtaining false answers is an integral part of these methods. Therefore, sensitivity analysis (an equivalent of the validation process for analytical methods) should be performed in order to determine the performance of these methods and their influence on the next step, that of making a decision (Chapter 6).

With the aim of fully understanding the processes of the evaluation of the evidential value of physicochemical data it is necessary to first understand the origin of these data. Therefore, some details concerning the analysis of glass, flammable liquids, car paints, inks, and fibres for forensic purposes are presented in this chapter. The data obtained in the course of these analyses are used later in this book.

1.2 Glass

Glass is a material that is used in many areas of human activity. In domestic and commercial construction it appears most frequently as window glass, whereas in automotive transport
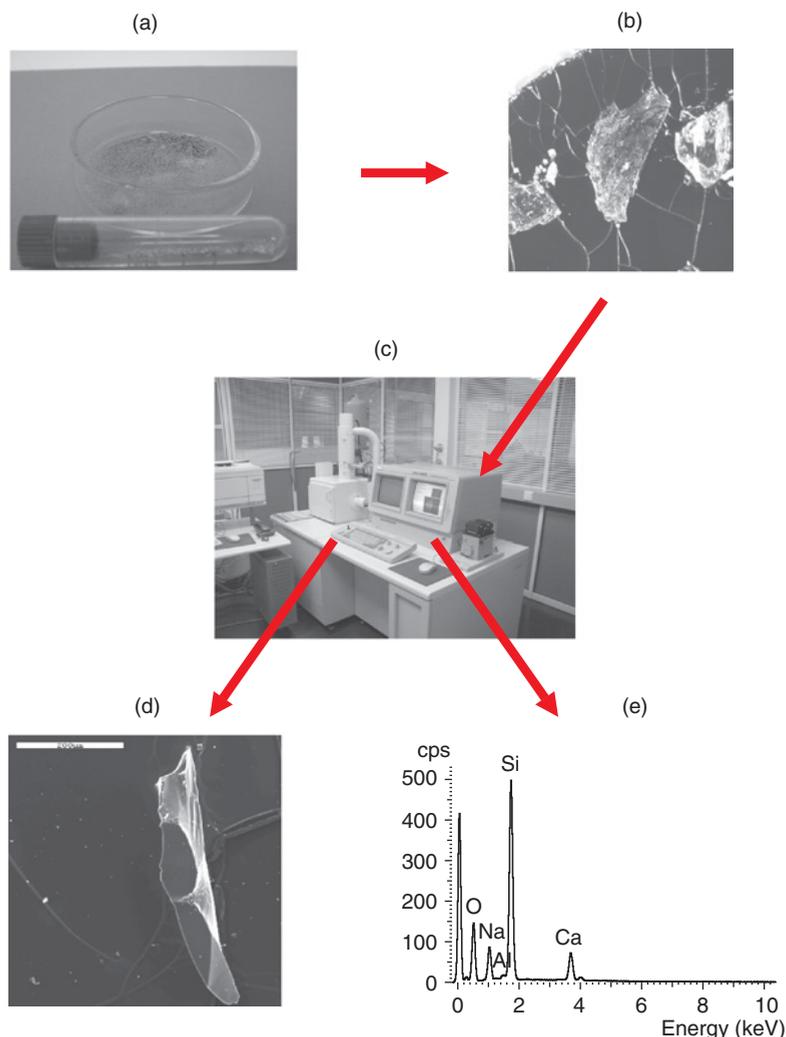
Figure 1.1 Principles of determination of elemental composition of glass fragments by the SEM-EDX technique: (a) debris collected from suspect clothes; (b) glass fragments located on an SEM stub; (c) view of SEM-EDX equipment; (d) SEM image of an analysed glass sample; (e) the spectrum of a glass sample obtained from an EDX detector.

it can form car windows and windscreens, car headlamps, car mirrors, and light bulbs. It is also used to make bottles, jars, tableware, and decorative items. Fragments of glass with a maximum linear dimension of 0.5 mm or less can be formed during events such as car accidents, burglaries and fights. These fragments may be recovered from the scene of the incident, as well as from the clothes and bodies of participants in any event of forensic interest (Figures 1.1(a),(b)). Such fragments may provide evidence of activity as well as the source of an object (Curran *et al.* 2000). The glass refractive index measurement (GRIM) method and scanning electron microscopy coupled with an energy dispersive X-ray spectrometer

(SEM-EDX) are routinely used in many forensic institutes for the investigation of glass and other trace evidence (Aitken *et al.* 2007; Evett 1977, 1978; Evett and Lambert 1982; Kirk 1951; Koons *et al.* 1988; Latkoczy *et al.* 2005; Lucy and Zadora 2011; Neocleous *et al.* 2011; Ramos and Zadora 2011; Zadora 2007a, 2009; Zadora *et al.* 2010; Zadora and Brozek-Mucha 2003; Zadora and Neocleous 2009a; Zadora and Neocleous 2009b; Zadora 2010).

Other methods used to determine the elemental composition of glass include: μ-X-ray fluorescence (μ-XRF) (Hicks *et al.* 2003) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (Latkoczy *et al.* 2005; Trejos and Almirall 2005a,b).

1.2.1   SEM-EDX technique

During the production of glass (Caddy 2001), many different elements are incorporated into the molten mixture. Certain elements are crucial for glass production and are always present. These major components are oxides of silica, sodium, calcium, magnesium, and potassium. Sodium is present, usually in the form of sodium carbonate, to reduce the softening point of silica, while calcium oxide and magnesium oxide make glass more chemically resistant. Minor components are also present, such as the oxides of aluminium and iron. Iron oxides are used to impart colour. Trace elements are also included, mostly depending on the required properties of the glass, particularly for any specialist uses.

The elements analysed using SEM-EDX are the major and minor elements found in glass. SEM-EDX does not allow for determination of the trace elements. The presence of the major and minor elements does not have great discriminating power as they are commonly present in glass. The trace elements are often regarded as imperative for the discrimination of glass, and suitable techniques are available for analysing the trace elements such as μ-XRF and LA-ICP-MS. However, in the field of forensic science the available equipment must often be used for as many purposes as possible, therefore if the concentrations of the major and minor elements alone can give correct and reliable data to solve a comparison and classification problem (Chapters 4 and 5), then the SEM-EDX method would be sufficient and useful for this purpose. The difference in the concentrations of these elements present in the sample is likely to be small. Therefore a statistical approach is imperative to detect any significant differences in the amounts of these elements.

The first stage of the SEM-EDX is the use of a scanning electron microscope (SEM; Figure 1.1(c)), which gives detailed three-dimensional images of a specimen (Figure 1.1(d)). The SEM works by using a beam of electrons as the source of illumination. A filament (e.g. made of tungsten) provides the source of electrons. As the filament is heated the electrons escape (thermionic emission) and a high voltage is applied to accelerate the negatively charged electrons away from the positively charged filament. The electrons interact with the specimen (e.g. glass sample) in various ways.

The X-rays produced by the SEM can provide information on elemental composition (Figure 1.1(e)), which is of interest here. The X-rays collected from the SEM are processed by an independent instrument (detector). Nowadays, an energy dispersive X-ray (EDX) detector is commonly used. This analyses the energy of the X-rays. The detector for the EDX system relies on a semiconductive crystal.

Quantitative analysis by the SEM-EDX method requires a surface of the sample be flat and smooth. An embedding procedure in resin could be used for sample preparation. This process is rather impractical for very small glass fragments (e.g. with linear dimensions less than 0.5 mm). Therefore, a question arises: is it possible to obtain useful information for

forensic purposes when small fragments are prepared without the application of an embedding procedure for SEM-EDX analysis? The study of this problem was described in Falcone *et al.* (2006). The results presented by Falcone *et al.* showed that the accuracy and precision of the results (wt. % of SiO_2, Al_2O_3, Na_2O, K_2O, MgO, CaO, Fe_2O_3, Cr_2O_3) obtained for a non-embedded sample were not as good as those reported for an embedded sample. Nevertheless, this experiment does not reveal if data obtained for non-embedded glass fragments are reliable when solving comparison and classification problems, for example by applying an LR approach. It was shown that a simple procedure of glass preparation could be applied and this procedure allows the user to obtain reliable data for solving comparison and classification tasks for forensic purposes (Aitken *et al.* 2007; Neocleous *et al.* 2011; Ramos and Zadora 2011; Zadora 2009; Zadora 2010). In this procedure glass fragments were selected under an optical microscope in such a manner that their surfaces were as smooth and flat as possible (Figure 1.1(d)). A comparison of the elemental analysis results obtained for both embedded and non-embedded samples of glass standards (NIST 620, 621, 1830, 1831, USA) was carried out by one of the authors (unpublished results of validation process) with the aim of checking that such a procedure delivers useful information for solving forensic problems. The accuracy and precision of the results obtained for these prepared glass samples were the subject of analysis. Moreover, the results were used with the aim of solving a comparison problem by the application of the LR approach. No significant difference between results (accuracy, precision, and likelihood ratio values) was observed. It was concluded that the proposed method of small glass fragment preparation for SEM-EDX analysis (which excludes the embedding process) could be satisfactory for use in the forensic practice.

1.2.2   GRIM technique

Refraction is the phenomenon of light bending as it travels through a medium with a different optical density (Figure 1.2). The refractive index of a material is the degree at which a light wave bends upon passage through the transparent material. The light bends due to the change in its velocity as it passes from one material to a material of differing density (e.g. from air to glass). The speed of light is slowed as the light enters the denser material, and this causes the light wave to bend.
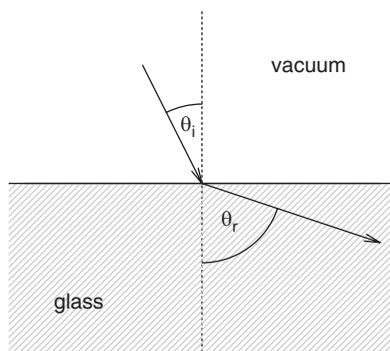
Figure 1.2   Refraction of light when passing from vacuum to glass.

A refractive index (RI) measurement quantifies the change in either the angle or the velocity of light and can be described by Snell's law:

$$RI = \frac{\sin \theta_i}{\sin \theta_r} = \frac{V_{\text{vacuum}}}{V_{\text{glass}}},$$

where θ_i is the angle of incidence, θ_r the angle of refraction, and V_{vacuum}, V_{glass} are the velocity of the light wave in the vacuum and glass material, respectively. The refractive index value effectively measures the ratio of the velocity of light in a vacuum to the velocity of light travelling within the transparent medium, or the ratio of the sine of the incident angle to the sine of the angle of refraction.

The RI of glass can be valuable as its value is affected by many factors. These include the substances present in the glass, the manufacturing process, any subsequent heating and cooling, and any stress present in the glass. This means that the RI value is highly discriminatory for glass samples and can be used for solving comparison problems (Chapter 4).

The RI of glass may be determined using a GRIM instrument (Figure 1.3(a)), which has a hot stage (Figure 1.3(b)) controlling the temperature of the immersion oil in which the sample has been mounted with a precision of at least 0.1°C. The glass fragment to be measured must be submerged in a suitable medium such as silicone oil. GRIM exploits the fact discovered
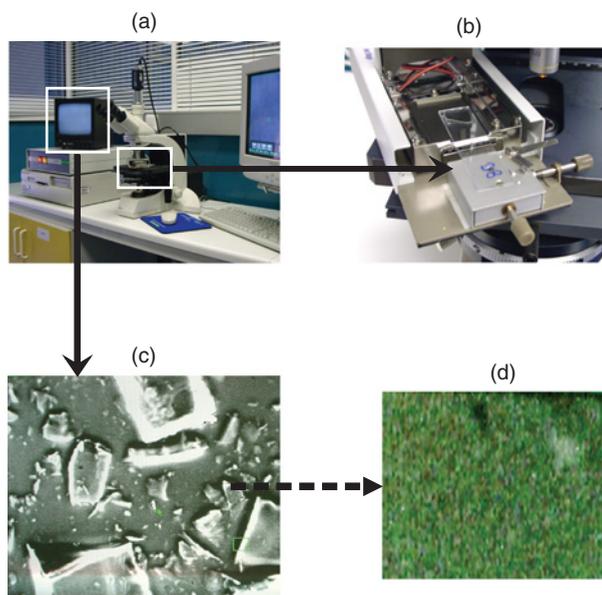
Figure 1.3   Principles of the thermoimmersion method for determination of the refractive index of glass microtraces: (a) GRIM2 set; (b) the appearance of a glass sample in an immersion oil on a microscopic slide located at the hot stage; (c) an image of glass edges in an immersion oil at the temperature when its refractive index is different from the refractive index of the measured glass sample; (d) an image of glass edges in an immersion oil at a matching temperature when its refractive index is equal to the refractive index of the measured glass sample.

by Emmons in the 1930s that the RI of a liquid varies with changing temperature, but the RI of glass changes very little with changing temperature. The technique also relies on the fact that when the RI of the liquid and the glass are the same the glass fragment submerged in the liquid is no longer visible (Figures 1.3(c),(d)). Therefore, when a piece of glass is submerged in a suitable medium (such as immersion oil) changing the temperature changes the RI of the immersion oil, but not the RI of the glass. The temperature can therefore be changed until the RI values are the same and the glass is no longer visible. In the past, the operator measuring the RI had to monitor for the disappearance of the glass fragment by eye. However, the development of the GRIM by Foster and Freeman meant that an instrument could replace the eyes of the operator. To do this the GRIM software identifies the point at which the contrast between the edge of the glass fragment and the oil is lowest and obtains a match temperature for this point. The match point temperature is then converted into the RI value by referring to a calibration equation which is compiled on the GRIM instrument using the same oil and reference glass fragments of known RI values.

   If the amount of material available for analysis is large enough (in general, objects larger than 0.5 mm), then the fragment can also be annealed (Figure 1.4(a)). During annealing, tensions present in the glass object (which have an influence on the value of the RI) are removed. Stresses occur in glass because of the limited thermoconduction of glass, which means that the outer glass layers can cool significantly faster than the inner layers. This effect leads to the establishment of internal stresses during the manufacturing process. The stresses take the form of compression in the outer layers, whereas the inner layers are subject to tearing forces. Annealing eliminates or reduces internal stresses in glass. The annealing process works in such a way that the stresses upon the inner layers are removed slowly during controlled heating at high temperature. Afterwards, slow cooling is carried out to allow the glass layers to relocate to those positions where the internal/external stresses are minimised. Commonly produced glass types such as building windows and container glass are most often annealed as part of the manufacturing process. However, some glass types are not annealed at all, and in others stresses are deliberately introduced as part of a toughening process (e.g. toughened glass used for car windows). During the analysis of glass objects, annealing is often undertaken in muffle furnaces (Figure 1.4(b)). The heating/cooling programme can, however, vary in a manner that is dependent upon the glass and the preferences of the individual scientist. A typical temperature programme (Caddy 2001; Casista and Sandercock 1994; Locke and
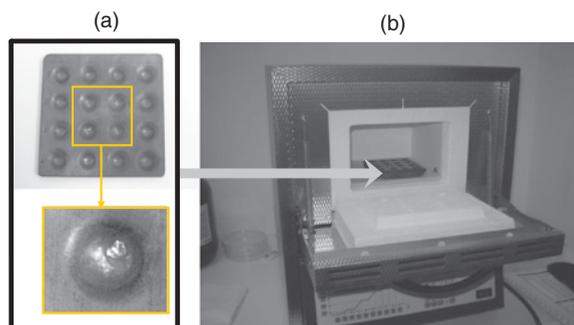

Figure 1.4 Annealing of glass fragments: (a) a metal holder containing glass fragments prepared for annealing; (b) a Nabertherm L3/11 muffle furnace with P320 programmer.

Underhill 1985; Newton *et al.* 2005) contains a step of fast heating up to 550°C, called the maximum temperature, at which most glass objects begin to melt. The glass fragment is kept at this temperature for some pre-determined period in order to eliminate the stresses present in the glass. In some laboratories, short temperature programs for annealing are conducted in tube furnaces. The glass fragment is heated up to 590°C, kept at this temperature for 12 minutes and then cooled at a rate of 4.5°C min^{-1} down to 425°C, at which it is held for 1 minute before being cooled down to room temperature (Caddy 2001). Long temperature programs, which employ fast heating of the specimen up to a high temperature, can be used, but this temperature is retained for a longer time (typically 10–15 hours) before the glass is slowly cooled down.

In general, for toughened glass, differences between RI values measured after annealing and those observed before the annealing process should be larger than differences for non-toughened glass. This is because of structural stresses introduced into the glass object during the toughening process, and removed during the annealing process. This information is used in a classification problem presented by Zadora and Wilk (2009) and in Section 5.4.2.

1.3   Flammable liquids: ATD-GC/MS technique

Arson is a frequently observed criminal offence (Almirall and Furton 2004; Mark and Sandercock 2007; Nic Daéid 2004; Zadora and Borusiewicz 2010). In cases where circumstances suggest that a fire might have been started deliberately, the scene of the putative offence is subject to the most comprehensive examination to recover materials and trace evidence possibly associated with the offence. The identification of flammable liquids, which can be used to start a fire, is one of the aims of forensic fire examination (Figure 1.5).

These compounds are flammable because they are volatile and they very quickly evaporate from the scene of the fire (Figure 1.5(a)). Success, therefore, depends on how quickly fire debris is collected, and how it is stored (Borusiewicz 2002). The correct method of fire debris packing and storage is in tightly closed metal cans, glass jars, or volatile compound free plastic bags. One of the difficulties in the interpretation of fire debris by gas chromatography (GC) is that the chemical composition of the debris often significantly differs from the composition of the primary liquid. This is because of partial evaporation, burning and contamination by pyrolysis products of other burned materials such as wood and carpets. Another problem is that volatile compounds are only present in trace amounts. Therefore, a desirable first stage of the analysis of flammable liquids in fire debris is the isolation and concentration of the traces of any flammable liquids which may be present in the sample.

Traditional methods of isolating volatile compounds in fire debris are based on procedures such as distillation and solvent extraction with substances such as carbon disulfate. These methods have been replaced by headspace isolation methods (Almirall and Furton 2004; Borusiewicz and Zieba-Palus 2007; Nic Daéid 2004; Zadora 2007b; Zadora and Borusiewicz 2010), in which a short tube packed with an adsorbent (e.g. Tenax TA^{TM}, Carbotrap 300^{TM}; Figure 1.5(b)) is inserted into a container with the fire debris. A fixed volume of headspace vapour is pulled through the adsorbent tube using a pump (dynamic headspace method), or compounds from the sample migrate to the adsorbent by diffusion only (static headspace method). The adsorbed compounds are then recovered either by solvent extraction or by heating (Figure 1.5(c)). A direct headspace method, based on the collection and direct dosage of the fixed volume of the headspace phase, is often additionally employed. Automated
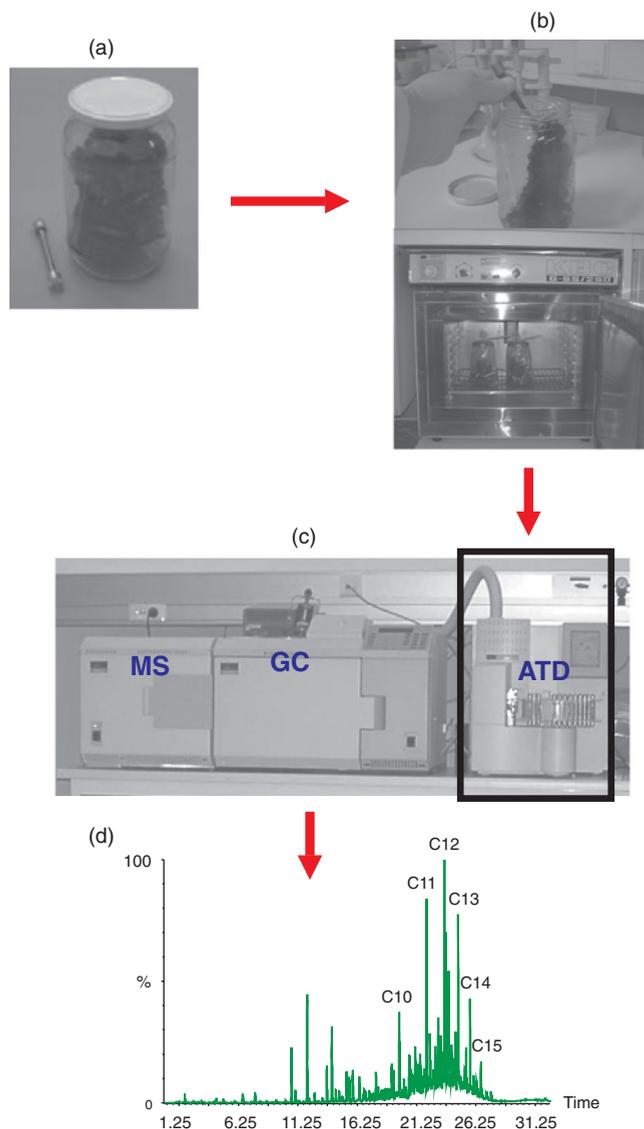
Figure 1.5 Principles of analysis of fire debris by the ATD-GC/MS technique: (a) a sample collected at the scene of a fire; (b) a metal tube filled with adsorbent Tenax TA^{TM} is placed in a jar with fire debris (top) and the jar put in an oven at a pre-concentration stage of analysis (bottom); (c) the metal tube is placed in the automatic thermal desorber (ATD; black box) and the sample analysed by the GC/MS technique; (d) a chromatogram, showing a result that allows the investigator to determine the category (here: kerosene) from which the flammable liquid originates.
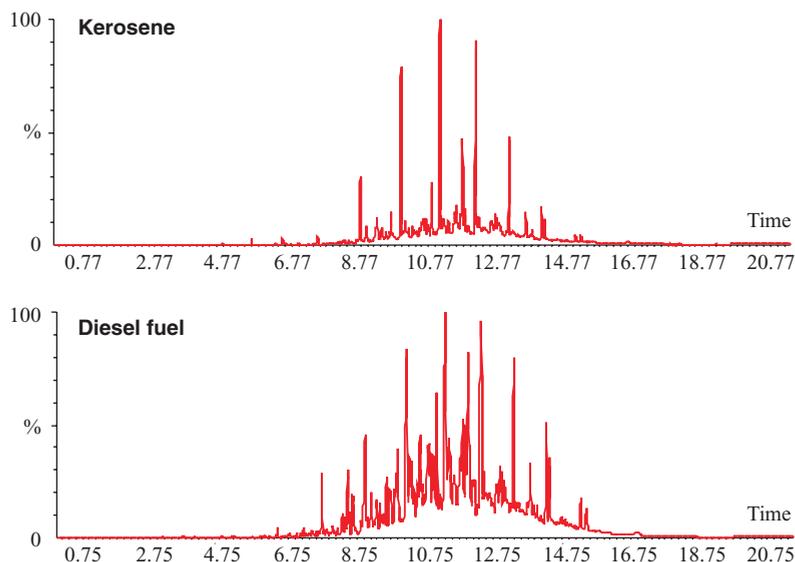
Figure 1.6 Chromatograms of partially evaporated samples of kerosene and diesel fuel revealing similarities in their composition.

thermal desorption coupled with a gas chromatograph (ATD-GC/MS, Figure 1.5(c)) is used as the secondary stage of analysis of fire debris separation on a capillary column. Nowadays, the detection of separated compounds is done using a mass spectrometer.

Flammable liquids detected in any fire debris sample require classification. Usually the American Society for Testing and Materials (ASTM E 1618) classification system is employed (ASTM 2006; Stauffer and Lentini 2003). The ASTM standard divides all ignitable liquids into nine classes: gasoline, petroleum distillate, isoparaffinic products, aromatic products, naphthenic paraffinic products, *n*-alkanes, de-aromatised distillates, oxygenated solvents, and a miscellaneous class of compounds. Each class, except gasoline, is in turn divided into three subclasses based on *n*-hydrocarbon range: light, medium, and heavy. Light indicates that the main components of the detected mixture fall in a carbon range from butane (C_4H_{10}) to nonane (C_9H_{20}), medium from octane (C_8H_{18}) to tridecane ($C_{13}H_{28}$), and heavy from octane (C_8H_{18}) to icosane ($C_{20}H_{42}$).

One of the analytical problems is the differentiation between evaporated kerosene and diesel fuel samples (Section 5.4.1) because there are only small quantitative differences in the composition of these chemical mixtures (Figure 1.6). The differences in their physicochemical properties result only from different amounts of individual components in each of these liquids. This is the reason why chromatograms obtained for weathered samples cannot be distinguished visually (Borusiewicz *et al.* 2006; Zadora and Zuba 2010).

1.4   Car paints: Py-GC/MS technique

The examination of car paints is an important aspect of road accident investigations (Caddy 2001; Thornton and Crim 2002). The paint fragments (Figure 1.7(a)) are collected from the
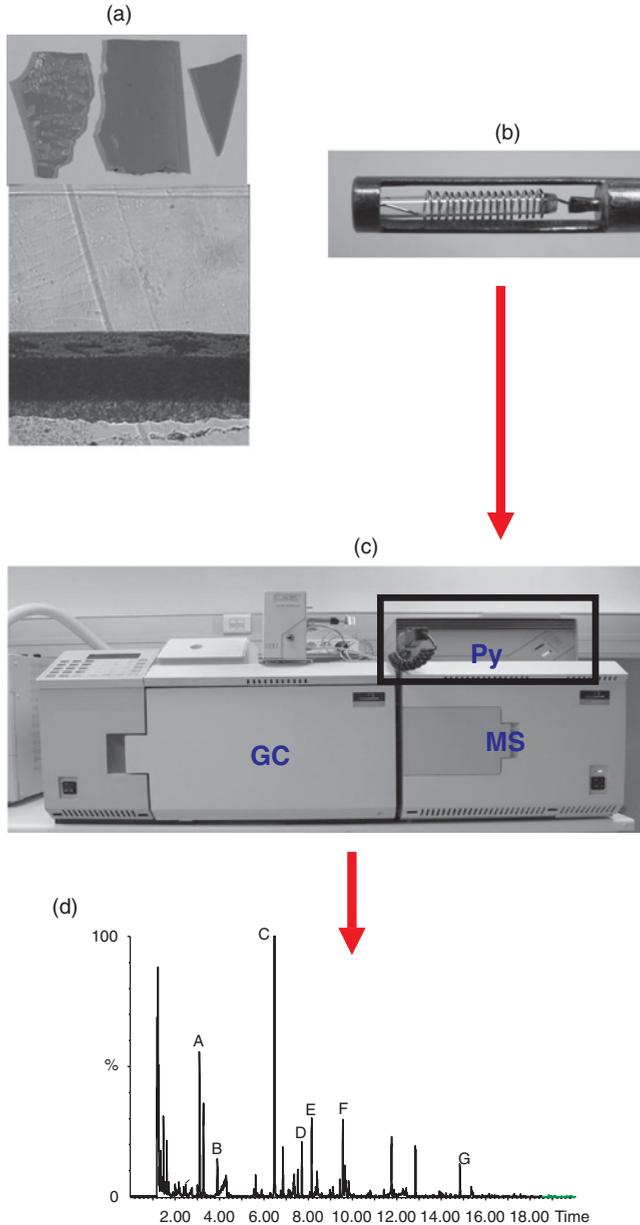
Figure 1.7   Principles of the analysis of car paints by the Py-GC/MS technique: (a) an image of a sample collected at the scene of a car accident (top) and a cross-section of car paint (bottom); (b) a car paint sample (50–100 μg) put into a quartz tube located in the platinum coil of a CDS Pyroprobe 2000 pyroliser (Py); (c) an analysis of pyrolysis products by GC/MS; (d) an example of a chromatogram obtained for styrene-acrylic-urethane car paint.

road or from the victim's clothes and compared with paint originating from the suspected car. It is necessary to obtain information on both the morphology and the chemical composition of the analysed samples in order to solve the comparison problem. First of all, different techniques of optical microscopy are used to define colour, surface texture and layer sequence (i.e. morphological features). Unfortunately, most paint specimens subjected to forensic examination do not have a full layer structure, so other methods have to be used to characterise the samples.

Fourier transform infrared (FTIR) spectroscopy (Zieba-Palus 1999) is an analytical technique routinely applied to determine the type of paint, that is, to identify the binder and the main inorganic pigments and fillers. Inorganic pigments can also be identified on the basis of their elemental content using X-ray methods, such as SEM-EDX (Caddy 2001; Zadora and Brozek-Mucha 2003). However, if the paint samples belong to the same class, that is, they contain a similar polymer binder and pigments, then further individualisation is required. This could involve the application of a more sensitive and discriminating analytical methods, such as Raman spectroscopy (Massonnet and Stocklein 1999; Suzuki and Carrabba 2001; Zieba-Palus and Borusiewicz 2006; Zieba-Palus *et al.* 2011) and pyrolytic gas chromatography mass spectrometry (Py-GC/MS) (Challinor 2007; Zieba-Palus 1999; Zieba-Palus *et al.* 2008a,b).

Py-GC/MS (Figure 1.7) has real potential as an analytical tool for the forensic examination of polymer traces (Caddy 2001; Challinor 2007). It can be used to characterise and compare polymer materials by determination of their components. During pyrolysis of the polymer sample (Figures 1.7(b),(c)), molecular fragments are produced which are usually characteristic of the composition of the original macromolecular material. The identification of pyrolysis products may be accomplished through the use of GC/MS. In paint examination, the chromatogram (Figure 1.7(d)) obtained may contain information about binder compounds as well as additives, organic pigments, and impurities, which could be helpful in the classification of paints or discrimination between paints with a similar polymer composition (i.e. similar FTIR spectra). Moreover, Py-GC/MS is able to find subtle structural or trace compositional variations within a matrix that is overwhelmingly similar among closely similar samples of the same category. The disadvantage of using Py-GC/MS for forensic purposes is that the sample is destroyed during analysis. It should be remembered that the analysed paint sample is the evidence and it should be available for various analyses at any time.

Numerous forensic applications have proved that Py-GC/MS can be used to characterise and compare polymer materials like paints by determination of their components (Milczarek *et al.* 2005). Several pyrolysis systems and techniques were discussed in overviews by Challinor (2007). Some authors tried to use this method for the discrimination between car paints (Burns and Doolan 2005a,b). Results presented in these papers showed that Py-GC/MS analysis confirmed results of FTIR spectroscopy analysis and allowed some subcategories within the particular category to be created. However, the distinction between samples of very similar chemical compositions remains a problem. An attempt was made to apply a likelihood ratio approach (Zadora 2010; Zieba-Palus *et al.* 2008b) to elaborate on the results obtained by Py-GC/MS analysis of styrene-acrylic-urethane paint samples that were indistinguishable on the basis of their infrared spectra and elemental composition (see also Section 4.4.3). The differences observed in the obtained pyrograms of the compared samples were small. Therefore, the aim of the analysis was to check if these samples could be distinguished on

the basis of selected variables, which represented the main compounds presented on their pyrograms.

1.5   Fibres and inks: MSP-DAD technique

Textile fibres (Figures 1.8(a),(b)) are useful pieces of evidence that can help to reconstruct criminal events (Robertson and Grieve 1999). Forensic fibre analysis is usually of a comparative kind, whereby a recovered sample is examined in order to determine if it could share a common origin with control fibres.

Inks are a type of evidence widely examined in the forensic field (Olson 1986). They are mainly collected in criminal cases such as suicides, document and will forgeries, and blackmail. In most cases such ink evidence is the subject of comparative analysis, the aim of which is to determine whether or not it shares a common origin with the ink sample from, for example, a document constituting a control material.

Microscopic observation (Palenik 1999) of the morphology is the first step in the analysis of fibres. It allows a screening of numerous items at the scientist's disposal and the selection of those that should be analysed further by instrumental methods. These include the characterisation of the polymer composition by FTIR spectroscopy (Adolf and Dunlop 1999; Kirkbride and Tungol 1999) and, for fibres which are dyed, a microspectrophotometric comparison of colour (Adolf and Dunlop 1999; Zadora 2010; Zieba-Palus 2005). Microspectrophotometry (MSP) in the ultraviolet and visible range (UV-VIS) or only in visible range (VIS, 380–800 nm) has been used as part of the analysis of coloured textile fibres in forensic fibre examination (Figure 1.8(c)), with the results being generated in the form of transmission and absorbance spectra.

One feature that differs among all objects is their colour (Shevell 2003). Colour recognition is only possible due to the eye's ability to receive light stimuli, which are further interpreted in the brain. Even though the human eye is considered to be a highly precise organ, each of us perceives the colours in a slightly different manner. Therefore, colour impression by the human eye has proved to be rather subjective and thus difficult for objective interpretation. Since the seventeenth century, when Isaac Newton explained the dualistic nature of light, the mathematical description of electromagnetic waves and colours has been a concern of physics. To eliminate such subjectivity, attempts at the standardisation of colour using a two-degree ($2°$) standard observer were introduced in 1931. The notation $2°$, along with the $10°$ observer introduced in 1964, refers to the viewing angle under which the standard observer sees the colour.

Comparison of spectra (Figure 1.8(d)) routinely involves overlaying these to determine whether they match with respect to peak positions and general spectral shape. Nevertheless, these approaches do not provide information about the value of evidence (Chapter 2).

An alternative approach which has been employed in forensic science (Olson 1986) is the parametrisation of MSP spectra in terms of coordinates in a three-colour system. The International Commission on Illumination (CIE) defines standards for colour transmittance, or reflection, for any illumination/measurement system, where a measured spectrum is weighted by some standard colour, under standard conditions. This is undertaken for the standard primary colours red, green, and blue, which are known as the tristimulus values of the *CIE XYZ* system. For microspectrophotometry with diode array detector (MSP-DAD) spectra, the
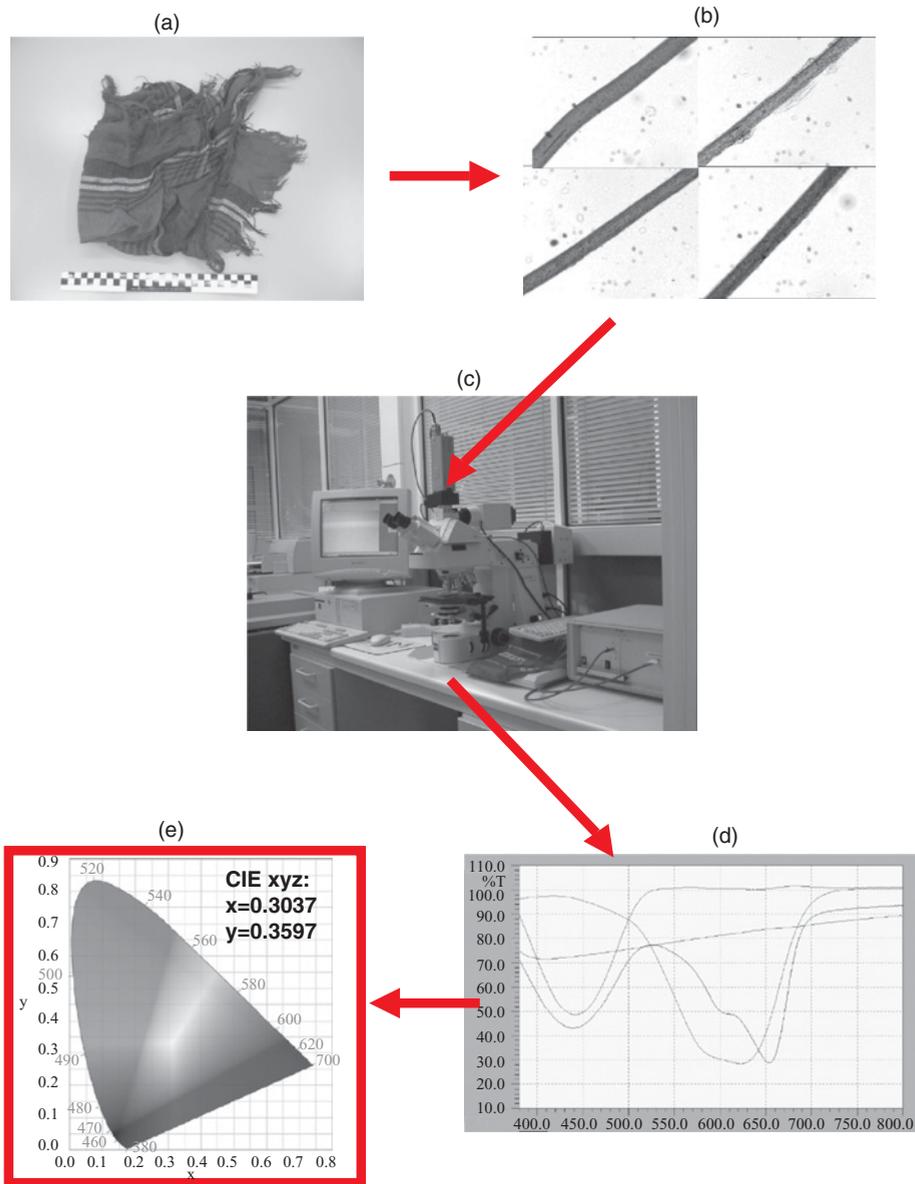
Figure 1.8 Principles of the determination of colour by the MSP-DAD technique – an example of fibres analysis: (a) textile sample; (b) fibre samples; (c) MSP-DAD set; (d) examples of spectra; (e) illustration of concepts of *CIE xyz* systems and an example of calculated chromaticity coordinates obtained for a fibre sample ($x + y + z = 1$).