Chapter 9 Chromatography

As has been shown in previous chapters, the identification of complex molecules is virtually impossible when only non-invasive methods are used. Such complex molecules are especially prevalent in organic materials, which unfortunately make up a large part of library materials. Many natural and synthetic organic materials can be found in library collections under numerous guises. Alongside 'traditional' materials like paper, parchment, papyrus, leather and ink, it is very common to encounter natural gums, waxes and resins as adhesives and surface finishes, and decorative and functional parts with dyed or printed textiles and binding media for paint pigments. Frequently photographs, prints and drawings of fine art, design and architecture make their way into libraries. By association with other objects through provenance and context, sometimes even furniture, sculpture and clothing enter library collections. The need and scope for identification of organic materials in library conservation therefore has a wide merit.

Two analytical methods are appropriate for the identification of organic materials described above. One is spectroscopy and the other is chromatography. Spectroscopy, as discussed in chapter 5 offers the non-invasive non-destructive techniques of ATR-FTIR, Raman and FORS options. These keep the physical structure of the object under study intact, with the added benefits of being portable point-and-shoot equipment at a relatively low-cost. Chromatography, on the other hand, normally needs a sample of material to be removed from the object. The sample is dissolved in solvent or pyrolyzed during analysis, making it unrecoverable. Access to expensive and specialised instrumentation and expertise is a further requirement. Why then even consider chromatography for library or museum objects? The decision whether to use chromatography rather than spectroscopy, comes down to the type of question that is being asked and the material that is studied. In essence, chromatographic analysis provides more chemical detail about a material than spectroscopy. Its strength for the heritage science toolkit lies in its ability to determine object authenticity and provenance and to assess material degradation.

Chromatography is based on the principle of separating chemical compounds in a material. Once separated, compounds can then be identified by coupling the chromatographic instrument to one or more sensitive selective detectors. Chromatographic analysis allows targeted analysis for specific individual compounds at nanogram levels (10^{-9} g) and below, with the compounds separated by subtle differences in molecular properties, for example the number and structural positions of hydroxyl groups. The outcome is a characteristic chemical profile for a material composed of one or more individual compounds that can be very similar or present in trace amounts. Chromatography is necessary when the level of specificity and differentiation can't be achieved by whole sample techniques like ATR-FTIR, Raman and FORS, which respond to molecular bonds common to related compounds without distinction between multi-component mixtures or degradation products. The conservation questions best answered by chromatography are those about the origin of a natural or synthetic organic material – its biological source or production, whether it is a mixture - and the causes and effects of degradation. Chemical separation is especially useful for identifying and studying the individual compounds of organic materials which tend to be multicomponent by virtue of inherent natural composition or synthetic production as well as a consequence of processing. A spectroscopic technique like ATR FTIR can readily differentiate a natural plant-based gum from a proteinaceous adhesive from an animal origin because gums are carbohydrates with no amide compounds. Likewise FORS can distinguish a plant red dye from an insect red dye when the visible absorption maximum is 560nm rather than 590 nm. Limitations are met in spectroscopy when the identification needs to be more specific – is the gum adhesive guar or acacia, or is the insect red dye source cochineal or lac? This specificity relies on distinctive 'marker' compounds and is where chromatographic analysis is a better choice than spectroscopy alone. It is these subtle differences

that are needed to establish the source or production of a material, or assessing the chemical changes to it from ageing and degradation.

Chromatography has been used for heritage science since the early 1950s, with one of the first published studies being paper chromatography for natural resins (Mills & Werner, 1952). From then came thin layer chromatography (TLC) for ancient adhesives and varnishes (Masschelein-Kleiner, Heylen, & Tricot-Marckx, 1968), gas chromatography (GC) for synthetic paint medium (Breek & Froentjes, 1975), high performance liquid chromatography (HPLC) for dyes (Wouters, 1985), size exclusion chromatography (SEC) for painting resins (de la Rie, 1987), pyrolysis GC for amber forgeries (Shedrinsky, Grimaldi, Boon, & Baer, 1993) and ultra HPLC (UHPLC) (van Bommel, Berghe, Wallert, Boitelle, & Wouters, 2007). Applications include TLC, HPLC and GC for textile dyes, inks and wood stains and for amino acids in proteinaceous fibres and adhesives. GC is common for oils, waxes, resins and gums in paint media, coatings and adhesives and additives like binders for paint and ink and plasticisers. Polymers are best done by py-GC and SEC. As the publication dates range indicates, the period from the 1970s through the 1980s was especially significant for the introduction of chromatographic methods into heritage science. Many chromatographic methods are now well-established for organic materials in historical artefacts. Chromatographic analysis plays an especially important role in qualitative and quantitative studies for heritage science, finding extensive application in studies of aged organic materials susceptible to chemical changes from reactions and interactions with environments - temperature, humidity, light and oxygen - that add to their chemical complexity and introduces variables. For most library collections the yellowing and disintegration of paper, fading of inks and dyes, and sticky release of plasticisers in plastics are unfortunately all expectations rather than possibilities. Chromatographic analysis has enabled chemical differences from these changes to be studied and compared for recreated materials, accelerated aged model and 'real-time' historical object to understand the cause and effect of degradation.

This chapter has a slightly different format than the previous chapters. Rather than dedicating one section to each individual technique, the first section deals with different methods for separating the compounds, while the second section deals with different detectors. The reason for this is that each method for separation has its own advantages when combined with a specific detector and in some cases detectors can be used on multiple separation methods. Anyone familiar with the chromatographic techniques of GC and LC will know the importance of the detection method otherwise the separated compounds cannot be identified. Key detection methods for chromatography are mass spectrometry (MS), ultraviolet -visible detection (UV/Vis), (photo) diode array detection (PDA, DAD), fluorescence (FL), and refractive index (RI).

There is much first-rate literature published giving specialist details for methodologies, technical improvements and new developments in heritage science and the related fields of analytical chemistry, biochemistry and forensic science. Current advances for historical material applications are mainly in methods of sample introduction and detectors with more statistical correlation of data to place an unknown in a material context by its spread of components rather than proscriptive specifics. Development continues to improve sample preparation detection and data interpretation through major research collaborations like EU-ARTECH, CHARISMA and IPERION.

For the purposes of this chapter, basic principles of established methods are presented to introduce concepts of chromatography rather than a comprehensive overview and critical discussion of all applications. Readers interested in more technical details will find several excellent reviews, books and conference proceedings well-worth consulting [Stuart, 2007; Colombini and Modugno 2009;

Degano et al. 2009; Artioli, 2010; Ferreira et al 2009; Madariaga, 2015; Mazzeo, 2017; Shahid et al. 2019).

Sampling

Choosing chromatography over spectroscopy for a library object usually entails taking a sample. Because removing material from objects is a major issue and involves conservation and curatorial input, it deserves a little reflection before the technical details of chromatographic analysis are discussed. A deciding factor for permitting sampling is the importance and necessity of gaining a high level of chemical information about the material. In other words, how much will the analysis benefit the object or a collection? Sampling for chromatographic analysis of organic materials in precious library objects is justified if there is good reason to understand the chemical composition of the material in detail. The binding media of illuminated scrolls (Lluveras-Tenorio, Mazurek, Restivo, Colombini, & Bonaduce, 2012) and the Seldon map (Kogou et al., 2016) and synthetic inks used by Van Gogh in his (Centeno et al., 2016) are some examples of library-relevant research involving chromatographic analysis of a sample, albeit very small, taken from objects. These multi-analytical studies demonstrate the powerful combination of spectroscopic analysis for general material identifications before chromatographic analysis, exemplifying why chromatography has remained relevant and appropriate alongside the introduction and development of non-invasive and non-destructive analytical methods. The principle of combining techniques starting with non-invasive 'screening' in as many relevant ways as possible before destructive analysis is well-established in heritage science. Good examples of it are multispectral imaging before dye analysis (Dyer, Verri & Cupitt, 2013; Dyer, Tamburini, O'Connell & Harrison, 2018) and the concluding study of the Monitoring of Damage to Historic Tapestries research collaboration (Odlyha, 2005).

The decision to sample is a responsibility often given to conservators with no sampling policy in place. To help navigate the decision process for analytical research, the Heritage Science Group of the UK's Institute of Conservation (Icon) has developed and published guidance. The Icon Ethical Sampling Guidance considers different circumstances for sampling framed by professional conservation (Quye & Strlič, 2019). The guidance centres around advantages and disadvantages of non-invasive and invasive techniques from the perspectives of the analyst and, importantly, the custodian of the object - conservators, curators, collection managers and owners – on a case-by-case basis. When a non-invasive method is unable to adequately answer the material question, sampling can be justified if it is permissible (Figure 3). Thinking about material analysis in an objective way helps address overly-optimistic expectations for high level specificity for material characterisation by non-invasive spectroscopy-based analysis. Portable instruments and the kudos of accessing prestigious objects or being 'scientific' with an easy to operate point-and-shoot instrument underplays the skill of the spectroscopist needed for result interpretation of complex historical materials and overshadows technical limitations of the methods. In such instances, ethical questions arise around the impact of not answering the research question fully or incorrectly.

Risks and Limitations of chromatographic Analysis

Dye analysis by HPLC and UHPLC, as well as TLC, all require samples of thread, yarn or fabric to be taken from the textile artefact, and the dye components to be extracted with solvents by a sample preparation step prior to analysis (Quye, Hallett & Herrero, 2009). Although the samples are destroyed by the analysis, the scientific value of the information gained for artefact provenance and colour damage risk predictions can be high enough for conservators and curators to permit sampling from discrete or damaged areas or loose ends of textiles. A single thread of 5mm length or a piece of fabric 5 mm^2 is adequate for HPLC and UHPLC (LC) analysis.

When sampling is necessary, analysts must weigh up expectations and limitations of analysis beforehand, and have enough specialised chemical and historical material knowledge to ensure quality data and meaningful interpretation. The analyst must also take responsibility for

documenting samples and sampling locations with unique identifiers, and undertaking or guiding sampling in person whenever possible, as well as keeping a detailed and secure record of the analytical method and data, reporting the results, and returning any unused sample to the collection. Useful guidance for sampling plans and sample documentation for material analysis in heritage science is given by the British Standard (British Standard Institution, 2012).

Where to sample from, how much is needed, what implement to use and how to contain the sample are four key questions for removing material from an object. Sampling from an object is a decision made between the analyst and the custodian. Choosing where to sample and establishing the minimum amount required, needs the analyst's input to ensure that the right sample for the question and technique are taken. Ensuring the safety of the object on both structural and aesthetic grounds, as well as the actual removal of the material is best done by a conservator, unless the analyst is particularly experienced in this field. It is important to discuss a pathway beforehand to ensure everything is prepared for sampling so that the task is done in a controlled and calm way. Some form of magnification under good lighting and means of photographing the object before and after sampling are often essential.

It is tempting to go for a loose or dislodged piece, but this might not be relevant for the question. It may not even have come from the object under study. Fibres and particles are easily transferred unwittingly, and dust can be misleading. Personal experience of dye analysis of loose textile fibres caught in the inside of an ancient Egyptian sarcophagus and finding it to be synthetic is testament. If the analysis is to identify the source of the material, a sample from the most protected part of the object is best, to minimise chemical change from environmental exposure. A bonus will be if this can be taken from a discrete area, which for a three dimensional object can be underneath, behind, or inside. This is more challenging for a two dimensional surface. It seems obvious that if the question concerns degradation and ageing, then this degraded areas is to be sampled. It might be expected that this will be easiest, but as this is usually the most exposed area, it can be the most difficult to take aesthetically.

Sample sizes vary from a printed full-stop sized sample for binding media analysis to an eye-lash length yarn for dye analysis. Sample sizes for gas chromatography are often a pin prick in size. Sampling tools include scalpels, fine-point tweezers, bow-sprung scissors and tungsten needles as well as magnification and good lighting. Containers should be of a suitable size and made of an inert, non-static material otherwise the tiny sample becomes frustratingly difficult to remove without risk of loss. Samples can be sandwiched between clean glass slides or enveloped in acid-free tissue or filter paper, and labelling is best done with pencil rather than ink to reduce chances of cross-contamination. Plastics with plasticisers become troublesome interferents in trace analysis, so polypropylene and polyethylene are best. Laboratory spaces for sample preparation and analytical equipment need to be clean, environmentally stable and dedicated to trace organic analysis. Samples needs to be assigned their own unique code, so that no mix-up takes place during and after analysis.

From a practical perspective, successful chromatographic analysis depends on three essential factors. Can the compounds of interest be mobilised with solvents or by heating? Are the analytical conditions good enough to adequately separate the compounds of interest? And is the detector sensitive or specific enough to detect these compounds? Sometimes there is no suitable method or reference materials in existence, and the resources to develop it outweighs the gains. This is why seemingly straightforward material questions cannot always be answered, which underlines that even the most sophisticated scientific technique cannot provide all the answers all of the time. Knowing the possibilities and limitations of chromatographic analysis is therefore useful for curation and conservation in libraries and archives.

Chromatographic analysis involves interesting challenging chemistry to develop the best experimental method and understand the results. Analysts need knowledge of material chemistry to select the right methodology for the material and the question being asked, and laboratory skills to prepare samples and references and to operate and maintain the sophisticated, sensitive and expensive equipment. Good communication and close collaboration with conservators and curators allow the right sampling location for the question posed to be selected, the taking of a minimal but adequate amount of sample, and the interpretation of the data for meaningful conclusions. Analysts tend to specialise in one chromatographic technique, like GC or LC, and develop expertise for specific applications such as paint media or textile dyes, with larger research groups offering a suite of techniques. It is important that analysts are not only expert in their technical skills but also responsible and well-informed about heritage science with conservation awareness to consider risks and benefits of analysis.

The aim for a good chromatographic method, is that each compound is to be retained long enough for small molecular structure variances between similar chemical compounds to be differentiated, and for all molecules of each compound to experience the same interaction so that they chromatograph as a well-resolved group and reach the detector at the same time. Successful chromatographic analysis for source identification of organic materials usually relies on distinguishing between compounds with very similar molecular structures. This can come down to the difference between the type or structural position of just one functional group or bond, which necessitates very careful development of the right chromatographic conditions to ensure satisfactory separation.

To make identifications by chromatographic analysis, the chemical profile of multiple compounds and their distinctive combinations for the unknown material are matched to those of known reference materials. In this respect, chromatography plays another valuable analytical role in heritage science, that of ensuring organic reference materials are what they claim to be. Indeed, chromatography is essential in commercial research and quality control for the manufacture of raw and processed natural and synthesised materials. Several major heritage science research projects have used chromatographic analysis for foundational rigorous studies to ensure organic reference materials are correct (Kirby, van Bommel & Verhecken, 2014; Quye, Hallett & Herrero, 2009).

Separating compounds

The theory of separation that applies to analytical chromatographic methods is that the compound molecules are 'mobilised' by a liquid or gas carrier over a stationary material that attracts the molecules to different extents by chemical and physical processes. The basics of this principle, whether liquid (LC) or gas (GC), is best explained by an everyday example when a water-soluble ink spreads on wet absorbent paper. In this situation, the ink compounds are dissolved in the water 'mobile phase' and carried over the paper 'stationary phase' by the capillary action of water. Say this water-soluble ink is coloured red. It might be a single red colourant, a mixture of reds or a combination of colours for a desired shade. If the ink spreads across the paper as one red colour, it is likely to be a one red colourant of one chemical composition. If the ink separates into two or more bands of red, our educated guess is a mixture of two reds with different chemical properties. If the ink separates into red and yellow components, we deduce the overall red colour is a result of two or more colourants mixed together.

How far each colourant travels and how much it separates from others in a mixture depends on differences or similarities between the molecular structures of the ink's colour chemical components. As they interact with the molecules of the paper stationary phase and the water mobile phase, and transfer in and out of porous parts of the paper's polymeric structure. These

immobilising interactions between the ink compounds and the two phases, called retention, are due to an effect called partitioning, a combination of weak molecular hydrogen bonding and Van der Waals force interactions and physical transfer by absorption and desorption with the stationary phase. To be fully chromatographed, the ink mixture needs all its coloured compounds to partition as efficiently as possible by the time the water mobile phase reaches the maximum length of the paper stationary phase.

We can make judgements about relative differences and similarities between the dye compounds depending on how well they separate, in turn waiting to their retention. If we see distinct and separate coloured bands, the mixture of compounds is said to be resolved. Resolved components signal compounds that have experienced different partitioning effects from weak bond interactions between their molecules, the paper and water. If there are two resolved red bands, the likelihood is that they are two chemical classes of red colourants with different fundamental molecular structures, say a mixture of azo and triarylmethane colorants for example. Resolved red and yellow components are likely to have different chromophoric functional groups (carbonyl, amino or sulfonic groups), lengths of conjugated chains (number of carbon atoms and/or nitrogen atoms with alternating single and double bonds between them) or type or number of colour-enhancing auxochromic groups (hydroxyls, methyls and suchlike). If a red colour remains at the spot where the ink was put on the paper, this indicates coloured compounds which are not very soluble in the mobile phase, in this scenario a sign that they are probably highly non-polar, perhaps with few hydroxyl or many alkyl functional groups. Our eyes detect the visible spectral consequences of the above molecular effects, but not the structural detail needed to specify chemical classes to pinpoint sources.

PC and TLC

The above scenario of ink separation with paper and water is a crude description of paper chromatography (PC). PC was one of the first published chromatographic applications in heritage science, the analysis, under controlled laboratory conditions, of natural resins in paintings (Mills & Werner, 1952). More advanced chromatographic techniques have been developed since then, but the relative simplicity of PC serves well to illustrate chromatography in general. For PC analysis, samples are dissolved in a solvent and spotted with individual fine capillary tubes or needles onto a pencil-marked line near the edge of the paper. The paper, loaded with samples, is then suspended in a shallow amount of mobile phase solvent inside a lidded glass container. Multiple samples can be loaded in a row on the stationary phase and 'run' simultaneously. The mobile phase can be a single solvent but is often a mixture developed to maximise the spread of the compounds of interest. The distance that each compound travels from its start position to its maximum is measured and ratioed against the distance of the 'solvent front', the maximum travel distance of the mobile phase (R_f) in the run time. Patterns of R_f for separated compounds are diagnostic for different materials.

Thin-layer chromatography (TLC) is an advancement of paper chromatography, with stationary phases of silica microparticles 10-12 μm in diameter bonded to a glass or aluminium plate. It was the dominant liquid method in the early years of chromatography until the 1970s when high performance liquid chromatography (HPLC) was developed. Early examples of TLC for heritage studies are identifications of adhesives and varnishes and proteins in paint binders using ninhydrin to visualise the separated compounds (Masschelein-Kleiner, 1974), plant gums (Kharbade & Joshi, 1995) and dye analysis (Taylor, 1992. The stationary phase for TLC analysis is pure or chemically-modified porous silica coated onto glass or a metal support (plate), and the mobile phase either a non-polar organic solvent or an aqueous or polar solvent. Like PC, the TLC plate is placed in a shallow amount of mobile phase in an enclosed tank. Useful TLC stationary phase options include polar silica or kieselguhr (a diatomite silica) with 'normal phase' mobile phase solvent mixes like hexane and chloroform for non-polar organic compounds such as waxes. Alternatively, 'reversed phase' (RP)

with non-polar alkyl compounds of 8 (octyl, C8) or 18 (octadecyl, C18) carbon atoms bonded to silica or kieselguhr with polar, often aqueous, mobile phases with high purity alcohols, chloroform, pH modifiers like ammonia, and salts can be used. RP-TLC was used extensively in heritage science from the 1970s to the 2000s for painting binding media (Striegel & Hill, 1997) as well as natural dyes and recently to isolate synthetic ink compounds in artists' felt tip pens with an innovative TLC stationary phase of silver iodide on gold-coated glass plates before identification by SERS and FTIR (Germinario, Garrappa, D'Ambrosio, van der Werf & Sabbatini, 2018).

TLC is efficient and relatively low-cost for common substrates and is very effective in expert hands. Despite its seeming simplicity, TLC needs great skill but even with continuing developments, its separation power is limited by being a thin layer of stationary phase. High performance TLC (HPTLC) with smaller 5µm silica particles and automation was introduced in the early 1970's and today is used extensively in industrial analysis to screen natural biochemical and pharmaceutical materials. (HP)TLC and meets limitations in the amount of stationary phase on the plate and the mobile phase moving by capillary action and hence a slow rate, while the size of the plate is a physical restriction for the amount of stationary phase and time for mobile phase to interact. Because it offers little advantage over TLC in heritage material applications and HPLC arrived about the same time as its introduction, HPTLC has had little application for library and museum material analyses.

HPLC and UHPLC

High performance liquid chromatography (HPLC) is an instrumental version of (HP)TLC that appeared in 1970 and now has countless analytical chemistry and biochemistry applications, especially in RP-HPLC mode (non-polar stationary phase, polar mobile phase). HPLC was applied to heritage science in the late 1980s, significantly by Wouters for historical and archaeological dye identification (Wouters, 1985). The stationary and mobile phases are similar to TLC with advantages of better separation and instrumental detection. HPLC has two major technical differences to TLC – a column to hold the stationary phase particles to increase surface area and hence contact time with the mobile phase, and high pressure to force the mobile phase through the column at a quick and constant rate. Efficiency of separation, direct instrumental detection and quantification of major and minor compounds are the major advantages of HPLC over (HP)TLC.

RP-HPLC stationary phases with C18 or C8, sometimes phenyl, are the most common and versatile library materials, and work well for polyaromatic compounds in natural and synthetic dyes, carbohydrates of gums, and amino acids derivatives from wool and silk fibres and binding media (Vanden Berghe, 2012) An amine-modified silica has also been used, for proteinaceous binders in manuscripts (Le Gac et al, 2013). HPLC silica stationary phases have smaller, more spherical particles than TLC, with3-5 µm diameter and pores averaging 7 µm rather than ranging up to 100 µm. These HPLC nano-particulates are tightly and densely packed into inert metal columns between 10 cm to 25 cm long with between 2.1 mm and 4.6 mm internal diameters. The physical properties of these tiny, uniform and multitudinous silica beads significantly improve chromatographic efficiency for superior separation over TLC because the efficiency of the stationary phase (N) increases as particle size (dp) decreases

$$1 dp$$

Invariably the 'real-life' materials of library and museum objects pick up unwanted non-polar residual compounds, i.e. dirt, that end up in the extracted samples and are indelibly attracted to the non-polar RP stationary phases with time, ultimately changing chromatographic behaviour and decreasing partitioning resulting in poor resolution. As the column is one of the most expensive regular consumables for HPLC, a short sacrificial guard column with the same stationary phase as the main analytical column can be attached to the inlet to prolong its operational life, although this increases the system pressure. For the mobile phase to pass through this packed HPLC column, a

high-pressure pump operating around 6000 psi is needed (for context, the squeeze of a blood pressure monitor cuff is just 3 psi) to achieve an optimum mobile phase flowrate of 1 – 1.5 mL min^{-1}.

Mobile phases for chromatography need to solubilise sample compounds and preferentially attract them from the stationary phase so that compounds are released within a reasonable run time, with 30-40 mins being acceptable. Common solvents for mobile phases, also called eluents, for library and museum material analysis by RP-HPLC are aqueous mixtures with polar water-miscible organic solvents. Eluents for RP-HPLC solvate compounds through hydrogen-bonding, dipole-dipole interactions and van der Waals forces, with water, methanol (CH_3OH), acetonitrile (CH_3CN) and tetrahydofuran (THF, $(CH_2)_2O$) appropriate for dyes, proteins and gums of historical interest. Modifiers like tetraammoniumbutyrate, formic acid, and buffers of citrate and acetate salts can be used to control and maintain pH for more efficient partitioning, although salts can be problematic for mass spectrometric detector. Regardless of the choice, solvents and modifiers must be free of impurities and additives for maximum sensitivity, demanding (expensive) analytical-grade chemicals and high-quality deionised and distilled grade water (18 MΩ).

There are two choices of HPLC mobile phase pump – isocratic and gradient. Isocratic pumps deliver either a single solvent eluent or one constant composition of a mixture of solvents/additives. Gradient pumps combine two, three or four different solvents to deliver a single mixed eluent of changing composition during analysis while maintaining constant pressure and flowrate. Solvents readily absorb air which prevents both types of pump from working properly, so they are degassed by vacuum or purging with helium gas. It is transferred from the pump to the sample injector, column and detector via narrow-bore 1 mm internal diameter stainless-steel or, if THF isn't used, plastic (PEEK) tubing. Viscosity can change subtly with room temperature, affecting flow and causing column backpressure and the instrument to shut down. Column heating is therefore common, usually to 40°C, to smooth out mobile phase viscosities from changing eluent ratios. Samples are introduced into the eluent dissolved in a solvent (explained below), injected into the mobile phase flow, typically in volumes of 10-50 µL. A Rheodyne injector system is used to minimise interruption of the mobile phase flowrate or pressure. Injection can be manual or by an autosampler. The solubilised sample flows through the column where the compounds are partitioned between the stationary and mobile phases, and the separated compounds carried by the mobile phase to the detector. The eluent with sample exits the detector and is collected in a waste container for disposal. Leaking joints between tubing and the injector, column and detector are the main practical issues.

Gradient elution is especially useful for multicomponent materials, allow fine-tuning of conditions to optimise retention, and therefore separation, of compounds. An example from our lab is a method for dye analysis that allows coloured polyphenolic compounds - flavonoids, anthraquinones, neoflavonoids, orceins, curcumins, berberines and indigoids - to be separated in a single run for each historical dye sample. Separation is achieved by a gradient pump and three eluents – 10% aqueous methanol (eluent A), 100% methanol (B) and 1% aqueous formic acid (C). The pump siphons off and mixes specific amounts of each prepared solution, held in separate bottles (reservoirs), to create a mobile phase of changing composition. The method we use starts with 80% A, 10% B and 10% C, after 40 mins end up at 0% A and 90%, with eluent C pumped at a constant 10% throughout. The change of %A and %B is ramped and stepped, for example at 14 minutes the eluent is 30% A, 60% B and 10% C. This composition is held for 9 mins before another ramped change. The increasing % of methanol gradually solubilises the less polar compounds. This allows, for example, flavonoids (polar) and indigoids (less polar) to be separated in one run and even two anthraquinones differing by one -OH group to be resolved. HPLC has been used to study dyed textiles in historical dyers' manuals in library special collections and archives to understand past dyeing techniques and colour production (Quye et al., 2003; Ortega Saez, 2018).

Developing short compositions and gradient programmes for different types of samples takes time so analysts share their methods. The one used at the CTCTAH was developed by conservation scientist colleagues at the University of Amsterdam (Serrano, van Bommel & Hallett, 2013). The natural materials encountered in historical library objects have much commonality with botanical and food so analytical protocols for published methods and new developments in these fields are a good starting point for new methods and for improvements and new developments. Much technical development is needed for HPLC to find the right combination of stationary and mobile phase to separate the compounds and, critically, to balance minimal dispersion of the compounds with maximise time for them to interact and partition between the stationary and mobile phases within a reasonable run time. The payback is the ability to chromatograph complex mixtures with slight differences in molecular structures and detect them at nanogram levels (10^{-9}g). An example of library applications of RP-HPLC has been the differentiation of gum binders in illuminated Portuguese charters manuscripts (Le Gac et al., 2013). Resolution (R_s) depends on stationary phase particle properties (N, a measure of plates), separation of compounds (α) and compound retention time (k)

efficiency selectivity retention

Ultra high performance liquid chromatography (UHPLC) is a recent development of HPLC, introduced in 2004 for biochemical application and historical dyes in 2007 (van Bommel, Vanden Berghe, Wallert, Boitelle & Wouters, 2007). Using even smaller non-porous particles than HPLC, UHPLC increases resolution and shortens retention times to allow lower quantities of compounds in the order of picogramme (10^{-12}) to be efficiently chromatographed and detected. The attraction for heritage science is the requirement of smaller samples and UHPLC has been used to study textiles in dyers' pattern books in library special collections and archives (Wertz, 2016) (see case study below). The greater partitioning efficiency of UHPLC is achieved with narrower tubing and column diameters than HPLC, made possible by a new generation of RP modified silica stationary phases made of bridged ethyl-siloxane/silica hybrid material with minimal porosity to be stronger to withstand the great pressures exerted - up to 15,000 psi. UHPLC mobile phase flowrates reduce considerably, down to 0.2 mL min^{-1}, so there is significantly less solvent for a 40 min run making it more economical and ecological than HPLC. UHPLC equipment looks and operates the same as HPLC except with far narrower tubing, so needs more care and attention with extra filtration of samples and solvents to remove particulates and stray fibres that easily cause blockages and overpressure the system. HPLC methods can be converted to UHPLC but reference data for identifications need to be re-run because compound retention times and orders change and more compounds are detected by UHPLC. UHPLC equipment is more expensive than HPLC but is gradually finding its way into more heritage science labs as older HPLC equipment is replaced, although exchanging an HPLC chromatograph connected to an existing mass spectrometer with UHPLC isn't straightforward because the high pressure difference is problematic.

SEC

Another useful LC technique for heritage science, around since the 1980s, is size exclusion chromatography (SEC), also known as gel permeation chromatography (GPC). It is used for the analysis of macromolecules in polymeric materials like paper, parchment, textile fibres and plastics (Hallett & Howell, 2005). One of the first SEC applications in heritage science was to painting resins (de la Rie, 1987). A major difference of SEC compared to other LC methods is that compounds are separated and differentiated by molecular size from diffusion differences into and out of a porous stationary phase made from polystyrene gels and deactivated silica to minimise secondary bonding interactions. The chromatograph equipment and eluents are the same as for HPLC, with stationary phases that are rigid and stable polymers with a known and controlled porosity range, such as polydivinylbenzene.

SEC has been applied to library materials to the measurement of degrees of polymerisation of paper cellulose – an indicator of degradation from shorter chains – an important factor before mass deacidification of wood pulp paper as a conservation treatment (Łojewski, Zięba, Kołodziej, & Łojewska, 2011). Polymers from small samples can be analysed so long as the sample can be solubilised. This becomes a restricting factor for SEC because aged polymeric samples can be very difficult, if not impossible, to solvate. SEC gives very useful information, including lignin from paper, but is also an involved process that needs to be justified when techniques like UV-visible spectroscopy and viscometry are viable screening alternatives (Łojewski et al., 2011).

GC

Gas chromatography (GC), or more correct gas-liquid chromatography, was developed in the early 1950s. Like HPLC it has remained a key organic material analysis method in many different fields. For library and museum materials, GC is the best identification method for the thermally-stable large organic compounds in resins, oils, waxes, fats, plastic additives and pesticides and has been applied consistently from synthetic paint medium in 1975 (Breek & Froentjes, 1975) to a recent application to dyes (Poulin, 2007). It has been especially useful for identification of oil glycerides in paint media, and able to partition isomeric compounds (same molecular composition and weight but different position of the functional groups and side-chains).

The principles of GC and LC are the same - sample compounds are carried by a mobile phase flowing over a stationary phase for separation relating to molecular structure differences and then detection. GC differs from LC by having a gaseous mobile phase of pure helium (99.99%) or another inert gas instead of a liquid, and the stationary phase is a thin layer 0.1 to 0.3μm of high boiling point polymer or liquid coating inside a glass capillary column. The column is very long (15 m to 30 m) and fine (0.2-0.4 mm internal diameter) with enough flexibility to be coiled, with care onto a metal support about 20 cm in diameter to suspend the column inside a heated chamber.

GC samples are heated so that volatile compounds are released for partition and separation on the column. Under changing temperature conditions, the volatile compounds experience vapour pressure differences between the stationary phase and mobile gas, and molecular interactions with the stationary phase coating. The stationary phase is traditionally a crosslinked methyl or phenyl substituted dimethyl polysiloxane, named to indicate the amount of substitution (5%-phenyl)-methyl siloxane, or a polyethylene glycol which is sensitive to oxygen degeneration, and the porous open later tubular (PLOT) columns. Volatile compounds move with the carrier gas through the chromatograph by fine stainless steel or copper tubing of 0.1 mm to 0.25 mm internal diameter (i.d.) and enter different temperature zones inside the instrument. Critical zones are the column oven which follows a precisely programmed temperature gradient between 50^{o}C and 350^{o}C for efficient partitioning and the injector which tracks the oven temperature to ensure all compounds are released and reach the column. The exit port post-column and pre-detector is held isothermally a few degrees above the maximum programmed column temperature 350^{0}C to ensure all volatilised compounds reach the detector. Carrier gas flowrate is programmed to change in the range of 2 mL min^{-1} to 10 mL min^{-1} during the run to maintain constant pressure when the oven temperature changes. Runtimes are similar to LC, around 30-40 mins per sample. Detection limits are in the order of 10^{-12}g.

There are three common ways to get the sample into the GC carrier gas. One is to inject the sample as pre-treated solvated liquid (discussed below) via a needle using a manual syringe or an autosampler that pierces a self-sealing silicone septum, or directly onto the column head with a fine capillary needle, called on-column injection. Another is to pyrolyze an untreated solid sample at temperatures around 500-600^{0}C in a special inlet, in split injector mode with carrier gas 'dilution' of

the sample if needed. This is called py-GC with a successful application being the characterisation of traditional East Asian handmade papers by triterpenes and phytosterol compounds (Han, Daheur, & Sablier, 2016). It is particularly useful on aged insoluble and/or cross-linked historical resins, binding medium, plastics, rubber or fibres. The third method, developed for air-borne volatile compound is to collect and trap the compounds on a very short length of GC-like capillary column (solid phase micro extraction (SPME)) or HPLC-like microparticulate column like Tenax (thermal desorption (TD)) and to heat this mini-column in the inlet to re-release the compounds. An example of the application of this technology is the study of pesticide vapours in museum stores (Rushworth, Higgitt, Smith, & Gibson, 2014) and a case study for modern paper given below.

Like LC, much research has gone into method development for different materials of heritage interest although it is fair to say that GC has fewer variables than HPLC or UHPLC and is therefore by comparison relatively more straightforward. From a practitioner's viewpoint, the instruments are easier to operate and maintain. Problem troubleshooting usually lies around loss of the stationary phase from repeated heating (column bleed), retained highly non-polar compounds in the injector and on the column head that are gradually released with repeated injections (carry-over), and overload if the sample compounds are in too high a concentration. Plasticisers from plastic are a common contaminant at the level of detection.

Ion Chromatography

The above-mentioned chromatographic methods are all for organic compounds, but there is one particularly useful LC technique called ion exchange chromatograph (IC) for soluble cationic and anionic inorganic compounds in heritage collections and spaces. IC has been applied to the quantification of air-borne pollutants in library and museums environments namely the acetate, nitrate, sulphate and chloride anions (NO_3^-, SO_4^{2-}, Cl^-) and calcium, sodium, and ammonium anions (Ca^{2+}, Na^+, NH_4^+) deposited on the surfaces of walls and showcases in archives using IC and inductively coupled plasma mass spectrometry (Skytte, Rasmussen, Svensmark, Ryhl-Svendsen, & Brimblecombe, 2017) and to inherent degradation compounds in cellulosic plastics (Mazurek et al., 2019; Quye, Littlejohn, Pethrick, & Stewart, 2011). The mobile phase is aqueous and can be a miscible organic solvent like acetonitrile or an aqueous soluble salt like sodium carbonate (Na_2CO_3) with sodium hydrogen carbonate (bicarbonate) $NaHCO_3$, or mixtures, and elution method gradient or isocratic. For anion analysis, a polymethylmethacrylate-based column with 6µm bead size is suitable, and capillary columns are available too. The quality of water for the eluent, especially the total organic content, is critical to successful analysis, affecting efficiency of partitioning and separation and detection background. Also, ionisable compounds are responsive to pH which influences ionisation states and solubilities and therefore needs to be controlled during analysis, which is a role of the eluent.

Detectors

The separation methods described above are only half of the chromatographic analysis story. Detectors are essential to differentiate separated compounds eluted from the column and to identify them by quantitative and qualitative measurement and comparison with reference compounds (see below). Detectors are coupled in sequence after the column so that the partitioned and (hopefully) well-resolved compounds pass to them. This is why chromatographic techniques are hyphenated, like GC-MS and HPLC-UV/vis.

The role of the detector is to stimulate, and respond to, the atomic structures of organic compounds. For example, a UV/Vis detector responds to the energy and wavelengths of visible and UV light and is ideal for (U)HPLC analysis of dyes and inks, and coloured compounds. An especially powerful detector for LC and GC is a mass spectrometer (MS) which fragments compound molecules into smaller parts, ionises them by bombardment with gases or liquids, then separates the

fragments by charge differences to measure their mass-to-charge ratio. Other more specialised detectors are fluorescence for (HP)TLC and (U)HPLC of chemically-tagged compounds, conductivity for IC and refractive index for SEC. These sensitive and selective detectors offer atomic-level interaction, and when combined with molecular-level compounds separated by the chromatographic system it is possible for compounds to be readily differentiated at the nanogram to picogram level (10^{-9} to 10^{-12} g). To ensure maximum sensitivity, it is essential that mobile phase solvents and chemicals and those for sample preparation (see below) are pure and do not compete or obscure compound detection. Detectors collect a large amount of data, generating large file sizes that need good management to ensure there is enough computer storage and that data is securely and regularly backed up.

UV/Vis, PDA and Fluorescence

Three spectrophotometric detectors are commonly used for LC analysis of a wide range of organic compounds: variable wavelength (UV/Vis); photodiode array (PDA, also called DAD); and fluorescence (FL). These very sensitive detectors expose compounds in the mobile phase to wavelengths of light as they pass through a flow cell with a small volume of 6.5 µL to 10 µL. UV/Vis and PDA detectors generate ultraviolet (UV) light from deuterium lamps (180 nm to 400nm) and visible light (Vis) from tungsten lamps (380 nm - 800 nm) to create wavelengths with specific energies corresponding to electron transitions in aromatic and aliphatic molecular structures with double bonds (-C=C-, -N=N-, -C=N-) and dipolar functional groups (>C=O, $>NH_2$), such as proteins in binding media and chromophoric (coloured) compounds in dyes and inks. FL detectors use deuterium or xenon lamps, as they require UV light only to excite inherently fluorescent compounds or derivatised with chemical markers that enhance their fluorescent properties (see sample preparation below) which the molecules respond to by emitting lower wavelengths.

During analysis, the wavelengths from the lamps are carefully controlled and precisely measured. UV/Vis and FL detectors pass their light through a 5 nm slit first, then either a rotating diffraction grating for UV/Vis or a monochromator filter for FL, so that compounds in the flowcell are exposed to selected narrow bands of specific wavelength. On the other side of the flowcell is a dedicated photodiode that responds to the intensity of light reaching it. If a compound absorbs a specific wavelength, the photodiode registers a weak signal that measured and converted to absorbance units (AU). The output from this sample photodiode can be compared at a near-continuous rate to the signal from a reference photodiode responding to light directly from the lamp for better performance. Wavelengths can be programmed to change during a run to maximise detection of chromatographed compounds with different absorbances or compensate for changing absorbances of a gradient eluent. UV/Vis detectors for IC operate at 200-220 nm, a useful range for anions and all organic acids. FL detectors are up to 100 times more sensitive than UV/Vis but not all compounds fluoresce or can be tagged, so applications are limited.

Photodiode array detectors are similar to UV/Vis detectors except the optics are arranged differently. Compounds in the flowcell are exposed to a slit-focussed full spectrum of UV and visible wavelengths from the light source that passes through the flowcell, which eliminates spectral distortions relating to changing mobile phase concentration. A grating on the other side disperses the light onto an array of usually 512 or 1024 individual photodiodes, each one controlled to respond to bandwidths of 0.5 nm to 1 nm and covering the whole spectrum of the light. The outputs from all the diodes is collated to provide a full spectrum of absorbances. For library and museum samples of dyes and inks, PDA is the best detector because if mixed coloured compounds are found, the data for all wavelengths has been collected and can be re-examined post-run for compounds that could be missed by a UV/Vis or FL detector if the right wavelength wasn't selected at the time of analysis.

FL detectors are best for tagged proteins in binding media and fibres. Important settings for detectors are response times (in the order of nanoseconds), and bandwidth settings to maximise selectivity to distinguish compounds (lowest, 1nm) or sensitivity to detect trace amount (highest,

8nm). Certain solvents and eluent additives absorb UV and have a cut-off wavelength below which compounds of interest are obscured, for example 215 nm for degassed methanol. Organic impurities in solvents and chemicals for mobile phases and sample preparation (see below) can also interfere and so must be of high purity.
Limiting factors for spectrophotometric detectors in heritage science are that similar molecular structures give similar responses, molecularly-different compounds can pass through the flow cell at the same time and their outputs superimposed, and unknown or unexpected compounds or degradation products cannot be identified without a reference. While satisfactorily confident identifications can be made with good reference materials, mass spectrometry is essential for the most rigorous degradation studies and identification of new compounds.

MS
Mass spectrometers (MS) are the most selective detectors for LC and GC and the most sophisticated, with instrumental variations for different applications, making MS an analytical specialism in its own right. Three of the most common MS modes for heritage science chromatography are: electrospray ionisation (ESI) in positive and negative ion modes to target different types of compounds; quadrapole ion trap techniques for multiple molecular fragmentation sequences for structural analysis (MS^n); and matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) for large molecules. Readers interested in further details and fuller range of MS possibilities than discussed here will find the many excellent publications for heritage science very useful. MS is essential for pyrolysis GC where the complex products from combustible and volatile compounds in a sample are analysed.
The fundamental principle of MS detectors for LC and GC is to measure very precisely the molecular mass, and hence structures, of chromatographed compounds. This is achieved by ionising compound molecules under carefully-controlled conditions, then separating them by their mass to charge ratio (m/z) and measuring this property. Ionised molecules can also fragment with or without intervention into smaller molecular structures in distinctive ways depending on their atomic arrangements, a diagnostically-useful property utilised to generate characteristic fragmentation patterns and compare them for identification. This makes MS highly selective for specific compound identifications than spectrometric, spectrophotometric and conductivity detectors alone but MS is less sensitive for trace compounds. To fully utilise the selectivity of MS and sensitivity of other LC and GC detectors, they can be coupled in sequence post-chromatography to create very powerful techniques that get the most information from one sample. Successful combination analysis relies on highly reliable instrumentation otherwise the sample can be lost if part of the equipment breaks down or physical connections fail. A dedicated experienced analyst also needs to ensure eluents and chemicals are compatible and interpret the results.

MS detection is a two-stage process. First, charged sample compound molecules are formed by ionisation then analysed with an electromagnetic field. The four most common ionisation methods for organic library material analysis are: electron impact (EI); electrospray (ESI) atmospheric pressure chemical ionisation (APCI); and matrix assisted laser desorption (MALDI). These ionisation methods are used with four common configurations of analyser – single and triple quadrupole, time of flight (TOF) and ion trap.

Let's consider the ionisation process first. Choice of the ionisation method relates to the molecular properties of the chromatographed sample compound. EI is common for the volatile compounds from GC, where the chromatographed compounds exit the column via a high temperature interface then enter a MS ionisation chamber where bombardment with an electron (e^-) beam (energy around 70eV) charges and fragments the molecules. A simple example is EI of methanol:

$$CH_3OH + e^- \longrightarrow CH_3OH^{+\bullet} + 2e^-$$
molecular ion

then

$$CH_3OH^{+\bullet} \longrightarrow \underset{\text{fragment ion}}{CH_3O^+} + H^\bullet$$

or

$$CH_3OH^{+\bullet} \longrightarrow \underset{\text{fragment ion}}{CH_3^+} + OH^\bullet$$

There are many applications of GC-EI MS by EI in heritage science, a few examples being: efflorescence on beeswax seals (Bartl, Trejbal, Ďurovič, Vašíčková, & Valterová, 2012); drying oils on paper (Banou et al., 2016); and degradation studies of dammar varnishes by indoor pollutants (Bonaduce et al., 2013).

For LC-MS of polar, non-volatile compounds in dyes, inks and proteinaceous binding media, ESI is common, often following UV/Vis, PDA, Fl or CI detection. ESI involves creating a fine spray of the chromatographed sample compounds in their eluent in the MS via a nebuliser before entering a chamber with a high voltage electrostatic field and a heated gas like nitrogen. This process forms droplets of molecular ions with single and multiple charges in a range of mass-to-charge ratios (m/z) of different relative amounts (abundance). These charged droplets are then attracted to a charged dielectric capillary where they are desolvated by the heated gas and the sample molecules reduced to microdroplets of charged ions. Fragmentation can lead to molecular rearrangement, and these molecules can in turn be fragmented in a multiple-step mass spectral process (MS^n). These charged 'parent' ions can be further ionised and fragmented with an inert gas, with the formation of negative ions best suitable to dyes (McNab, Ferreira, Hulme, & Quye, 2009; Troalen, Phillips, Peggie, Barran & Hulme, 2014) and organic inks [Centeno et al., 2016]. These charged fragments, called 'daughter' ions, also have characteristic m/z, like $-CH_2^-$ (m/z 14).

For samples with larger molecules there are two choices. APCI is suited to soluble polar compounds with some volatility, like fatty acids in oils and waxes (Saliu, Modugno, Orlandi, & Colombini, 2011) and phthalate plasticisers, involving vaporization at 500°C after nebulisation to form single-charged ions. MALDI, either under vacuum or atmospheric pressure, is suited to solid samples like collagen in medieval illuminated parchment scrolls (van der Werf, Calvano, Germinario, Cataldi, & Sabbatini, 2017), where the compounds of interest are ionised without decomposition by acid-base reactions using solvated matrixes and a pulsing laser, in this case α-cyano-4-chlorocinnamic acid and a Ng:YFG laser (345 nm) pulsing at 400 Hz.

ICP-MS ionises organic molecules and elemental compounds, for example the metallic compounds in dye mordants, with a plasma that draws energy from electrical currents formed by electromagnetic induction. ICP-MS can be used with GC and LC and in sequence after other detectors if needed. It has been applied to measurement of surface and sub-surface magnesium distributions in deacidified paper using laser ablation (Wagner, Bulska, & Sobucki, 2008).

Common analysers for GC-MS and LC-MS involve quadrupoles, a set of four metal rods with radio frequency and DC voltages applied between them. A single quadrupole analyser for GC-MS (with EI or chemical ionisation) allows ionised molecules with certain m/z to selectively travel the length between the rods if the voltage ratio allows them to, while those that don't have the right m/z collide with the rods, and by using selective ion monitoring (SIM), GC-MS 'fingerprinting' for quantification and identification, and while SIM for LC- ESI MS gives sensitive quantification but less successful fingerprints for identification. A mode offering high specificity and enhanced detection sensitivity for structural confirmations involves a triple set of quadrupoles, the first to filter ions of specific m/z, the second to fragment these ions by collision with a gas like helium or nitrogen and the third to scan or filter ionised fragments of specific m/z.

Another type of analyser, called time of flight (TOF), measures ion mass by accelerating ions down a tube using high voltage pulses and recording flight time (lower mass ions arrive faster) and relating this to the energy used for accurate mass calculations that can confirm structures. A fourth analyser is an ion trap, that uses magnetic fields to confine injected ions and then fragment isolated ions of selected mass and polarity by collision induced dissociation (CID), repeatedly if desired, until

detection. Multiple analysers can be arranged in tandem (MS/MS) for LC and GC, for instance TOF combined with quadrupoles (Q-TOF) and with MALDI for very fast scans.
For library-relevant analyses of dyes and organic inks, ESI is the most common mass spectrometric method. Certain molecular structures fragment in predictable and characteristic ways, resulting in spectra with distinctive fragmentation patterns (Figure 1).
Connecting MS detectors to GC is more straightforward that connecting them to HPLC and especially UHPLC because of the (ultra) high pressures of the liquid mobile phase and the need for compatible solvents.

Conductivity, Refractive Index and Light Scattering Detectors
A conductivity detector is used for IC analysis because the chromatographed ionic compounds have no chromophores and therefore no response to spectrophotometric detectors. The detector measures the conductivity of flow cell contents with two electrodes place one on either side. The conductivity measurement is made of the mobile phase rather than the separated ions. Buffers tend to be used for IC, so to suppress their response, a membrane of ion exchange resin, rather like those used for deionised water, can be placed between the column exit port and the conductivity detector to reduce the conductivity signal from the eluent and enhance the signal from the ions of interest. For example, in the analysis of anions, the suppressor would be a cationic material that contributes H^+ ions to neutralise OH^- and carbonic acid derivatives (HCO_3^-), thereby increasing the signal from the anion. For cations, the suppressor would be an anion with OH^- contribution.

Refractive index (RI) detectors are commonly used with SEC as a measure of absolute mass from optical refractive concentration difference between the sample and eluent and the eluent, measured by comparing refracted light from a tungsten lamp after passing through two flow cells, one for the sample and one for a reference of mobile phase. RI detectors need careful calibration to give meaningful results. SEC analysis of paper polymers has been performed using a UV/Vis coupled to an RI detector. An advantageous alternative to RI is the evaporative light scattering detector (ELSD) which nebulises the sample in the mobile phase in the presence of nitrogen gas and the suspended particles scatter light which is detected by a photocell. A version of ELSD called MALLS uses a multi-angle laser at 658nm combined with UV-Vis at 254nm to measure particles suspended in solution rather than a gas. Described as a detector for absolute mass, MALLS is influenced by sample concentration, refractive indices of the solvated polymer and detector operation parameters, which all need to be carefully factored in to the interpretation of the results. The principle of MALLS is the relationship between light scattering and polymer size expressed as molar mass. (Łojewski et al., 2011).

The chromatographic analyses described above take less than an hour to perform from sample injection to compound detection, but there are two additional essential steps which are highly skilled and time-intensive: sample preparation before analysis, and data interpretation afterwards.

Sample preparation
Choosing the right chromatographic and detection method is obviously key to successful analysis, but an essential step not discussed yet is preparing the sample for analysis. Sample preparation is important for several reasons. A fundamental one is that samples from library artefacts are normally solids and the types of materials they are made from needs LC and GC analysis. Samples thus need to be made soluble (dyes, polymers, soluble ions), volatile (resins) or tagged to be detectable (proteins in binding media) unless they are analysed by py GC-MS or collected by SPME. Sample preparation also extracts the most important compounds to answer the material question and/or maximises detection of trace amounts of compound by concentration. Another advantage is to remove interfering or dominant compounds in the material that obscure the compounds of interest.

Sample preparation starts by examining each sample carefully, typically at x20 to x50 magnification with a Dino-Lite™ or similar to photograph and document common and/or unusual physical features, including homogeneity. Sample preparation requires careful selection of solvents and chemical agents and requires much research time in the lab to optimise the conditions and best choice of solvents and agents to get the compounds of interest into solution. For chromatography, it is most efficient as a batch of samples, in the CTCTAH lab at the University of Glasgow typically 7 at a time with the 8th a reagent blank. The whole process for this sample preparation takes the best part of a day, involving documenting each sample by the code assigned at time of sampling and using this consistently and precisely in sample and result documentation, and labels for glassware and containers to track it during analysis. Mobile phases and extraction solutions need to be prepared. All reagents and solvents need to be of analytical grade with minimal contaminants and particulates, and high purity deionised and distilled water, and analysts need a clean dedicated lab with a fume cupboard. This requires the analyst spending several uninterrupted hours in a chemistry laboratory dedicated to micro-analysis, whether preparing one sample or a batch (in our lab up to seven samples plus a reagent blank at one time).

Different approaches are taken for different types of sample and the chromatographic method used, with variations between labs worldwide. The following are selected examples with brief descriptions to illustrate a wide range of practices. The references scattered throughout this chapter give more details.

Dyes

Dyes are normally analysed by LC and so need solubilised in a solvent. Analysts have a choice of extraction methods developed over many years through extensive research to meet the challenge of historical dyes from a wide range of natural and synthetic source – mordant, acid, basic and direct. They are removed from fibres by disrupting bonds with solvents, pH or acidified solvent for dyes mordanted with metallic salts. The longest established and most widely-used method is acidified aqueous methanol (Wouters, 1985), although it is known to chemically modify and potentially degrade certain compounds. Dimethyl sulfoxide with oxalic acid (Serrano et al, 2013), pyridine, water and oxalic acid (Mouri, Mozaffarian, Zhang, & Laursen, 2014), formic acid in methanol (Zhang & Laursen, 2005) and citric acid (Ford, Rayner & Blackburn, 2015) are now being used. Samples are individually heated in small vials with 100-200 µL of the solvent of choice between 60^{o}C and 80^{o}C for 10-15 mins, centrifuged and/or filtered to remove fibres and any particulates, and then the solvent removed by vacuum evaporation or under a steam of nitrogen. This dried extract is then reconstituted with 50-100 µL of a solvent appropriate to the mobile phase and transferred to a capped micro-vial stored away from heat and light until analysed.

Proteins (binding media, adhesives)

To solubilise animal adhesives on the Seldon map, sulphur oxidation, a carbonate buffer and a protein-digesting enzyme with mild heat and ultrasonication was needed. The whole process took about 12 hours. Pre-derivatisation is also possible, with a commercially available kit called AccQTag being used (Waters Inc.) which forms stable fluorescent derivatives with amines using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in 10 mins. This has been used to study the degradation for wool textile fibres in tapestries (Vanden Berghe, 2012).

Gums (adhesives)

GC-MS analysis of gums on manuscripts has involved hydrolysis with aqueous acid and pyridine and derivatised with methoxylamine using microwave assistance and derivatised to be volatile with N, O -bis(trimethylsilyl) trifluoroacetamide (BSTFA) with and without 1% trimethylchlorosilane (TMCS) (Lluveras-Tenorio, Mazurek, Restivo, Colombini, & Bonaduce, 2012). Gum arabic as little as 100ng, has been enzyme digested with exo-β-1,3-galactanase in a phosphate buffer for MALDI-MS and

derivatised for MALDI-TOF MS with 3-aminoquinoline in aqueous solvent. It was found that minute amounts of sample needed less enzyme and digestion times to improve the MS response, with the advantage of digestion directly on the sample to minimise loss and for reproducible enzyme hydrolysis profiles (Granzotto & Sutherland, 2017).

Paper
Polymer analysis of paper by SEC requires the cellulose to be dissolved, with two choices available – using dimethyl acetamide with lithium chloride additive (LiCl/DMAc) or using phenyl isocyanate to derivatise cellulose to cellulose tricarbanilate. It is not straightforward, though. Aged samples cross-link and this is a challenge for sample preparation for aged library materials. Because cross-linking occurs in aged polymeric materials, this gives rise to analytical variations that manifest in the different methods of extraction for SEC analysis by different laboratories, so a study was undertaken to compare the results (Łojewski et al., 2011)

While extensive application of chromatography to library and archival collections is limited by sampling needs, the technique is still worth knowing about because it is key in heritage science research of organic material degradation. Organic materials are sensitive to degradation from light, heat, humidity and pollutants even in the controlled environments for display, storage and study of libraries, archives and museums.

Reference materials are essential for identifications and need to be prepared too. Individual compounds can be purchased from commercial chemical suppliers, although impurities, expense and rarity are common issues for historically-relevant ones as many are of little commercial value or interest today. Individual compounds are not enough on their own for reliable identification. The characteristic chromatographic profiles of known dyes, gums, resins and other natural materials of specific botanical species must also be analysed as references. Some historically relevant synthetic dyes are still available, mainly as histology stains for medical and forensic pathology (Cooksey, 2017), although more needs understood about compositional differences between modern and past synthesis to ensure equivalence and relevance. Using natural dyes as an example, those of historical significance are derived from certain species of plants, wood, galls and scale insects, so those for analytical references must come from known and correct biological sources. These are best provided by botanical gardens and academic experts in natural history or through specialist commercial companies, not craft and hobby suppliers where biological origin details can be unintentionally obscure or inaccurate. To dye a textile with natural dyes, the compounds need to be extracted from the source. There are more in the dyestuff extracts than end up on the textile, so analytical references involve dyeing followed by sample preparation in the same way as the historical samples (Quye, Hallett & Herrero, 2009). Modern references can never replicate past materials and methods, so are treated as guiding models.

Interpreting Results
Results for HPLC, GC, SEC and IC are detector responses converted by software calculations into one or more type of graph. The graph that is common to all chromatographic analysis is the chromatogram giving an overall picture of the number of separated compounds detected and how well the compounds are resolved in the analytical run. The chromatogram shows a series of peaks of varying heights relating to the intensity of the detector response (Figure 2).

Chromatograms have an x axis of time and a y axis of the relevant response (Table 1). The x-axis is the retention time (t_r or rt) calculated from sample injection (t_0) to each detector response and measured at the peak apex, which corresponds to how long compounds are retained by the stationary phase. The y-axis is the relative intensity of the detector response. The height of each peak relates to the amount number of molecules, atom or elements detected, commonly referred to

as amounts, though technically incorrect, unless peak areas (calculated by the detector software) are quantified from known concentrations of the same or similar reference compounds. With UV-Vis and multispectral PDA detectors, a selected single absorbance wavelength, usually between 220 nm and 450 nm, is used. The retention order is important too, for example in RP-HPLC, polar compounds have shorter retention times than non-polar compounds. Typical total dye analysis times for UHPLC and HPLC are between 30 and 40 minutes.

Table 1 The y-axis of chromatograms and units by different GC and LC detection methods

Chromatographic method	Detection method	Chromatogram y axis	Unit
FL	UV/Vis	Spectral emission at one selected excitation wavelength	Fluorescence intensity
GC, LC	MS	Total ion count	Relative Abundance
HPLC	UV/Vis, PDA	Spectral absorbance at one selected wavelength	AU
IC	Conductivity	Electrical conductance	$\mu S\ cm^{-1}$
ICP	MS	Counts per second	Intensity/counts s^{-1}
SEC	RI, ELSD	Molar mass	kDa

Additional data from detected compounds adds confidence to identifications using the chromatograms. PDA and MS detectors can generate a second graph, called a spectrum, for each individual compound during the chromatographic run. This additional data from PDA enhances selectivity for coloured compounds like dyes (wavelengths on the x-axis instead of t_r). Spectra from MS detectors have an x-axis of m/z, and the selectivity of this detector makes it the best choice for organic compound for identifications and for studying new compounds like degradation products. The two case studies below demonstrate the power of these detection methods for chromatographic analysis of library materials.

Narrow and sharp means quick and efficient partitioning, which is typical for GC and HPLC chromatograms. SEC chromatograms are by nature broad peaks, being distribution curves of different molecular weight polymer chains. For successful identification, chromatographic peaks need to be resolved. If peaks are very close together or overlap because two or more components have similar partition properties and co-eluted, passing through the detector together, accurate and confident identification is hampered. Narrow spectral bandwidths for UV/Vis and PDA detectors can improve resolution, and detector software can be used to deconvolute them, although this carries the risk of mistaken assignment. It is better to change the chromatographic method to improve resolution. Peaks also need to be a minimum height to be detected and differentiated from background ‘noise’ of the detector and any contaminants or interferents. Ideally the baseline of the chromatogram should be a flat, but usually there are responses from organic solvents or other additives in LC mobile phases and chemical bleed from septa materials in the injector port or old column stationary phases in GC are detected, and manifest as sloped baselines in chromatograms. This is why it is important for chemicals and equipment materials to be of high purity. It is also possible for compounds from previous samples to be carried over into later analyses if the compounds are very slow to partition and released after the run time, another good reason for a guard column to trap them. In LC analysis, the first few minutes of retention time in the chromatogram is the mobile phase ‘solvent front’ response, so the peaks need careful interpretation.

In theory, all sample compounds should elute from the stationary phase. In reality, non-polar impurities in sample extracts and mobile phase bond to the stationary phase and change the

separation effects, while particulate contamination block the solvent flow, causing pressure build up and the equipment to malfunction with sample loss. Columns need to be changed periodically, but identifications from slightly differences in chemistry between each manufactured batch of stationary phase changes their chromatographic behaviour which affects r_t. This is a risk factor in historical dye analysis with precious single samples, so analysts must ensure good laboratory practice, and research and use the best analytical conditions.

Analysts use peak shapes to ensure their methods are appropriate and equipment is functioning. HPLC peaks broaden from inefficient partitioning, poor resolution between closely related compounds or a leaking connection in the equipment (GC peaks broaden too when seals fail). Curved or flat-top peaks indicate the sample is too concentrated, while lots of peaks in the baseline indicate dirty parts in the detector (like the flow cell in a UV/Vis detector) or trapped air in the eluent. A steadily rising baseline is a sign of chemical breakdown of the stationary phase or the silicone injector septum, or a build-up of unwanted non-eluting compounds (which guard columns can trap). Such things happen to the best analysts, but if they become the norm, then changes need to be made to the method or the equipment. Method development, careful preparation and good equipment maintenance are worthwhile investments.

Most of the time and effort of chromatographic analysis goes into data interpretation. The whole process from extraction to interpretation normally takes at least one day for an experienced analyst, longer if the sample is complex or unusual. To identify an unknown material, the rt and UV-visible and/or mass spectra of individual characterising compounds need to match the results of references for known compounds and materials. Organic material groups are defined by common molecular structures, for examples, carbohydrates for gums, terpenoids for resins, fatty acids for oils, polar polyaromatic compounds for dyes and repeating long chains of amino acids for protein fibres and plastic polymers.

Individual compounds and distinctive profiles of multiple compounds in the material are then identified by matching their response to those of reliable references for natural or synthetic materials, or known chemical changes caused by ageing or degradation. Identifications are based on retention times and by comparison of the sample chromatogram with the chromatograms of references of known compounds. This is reasonably straightforward for IC, where a small number of specific anions and cations can elute with consistent t_r under the same analysis conditions and for SEC with reference polymers of known molar mass. MS with SIM can identify specific molecular ions but is limited for unknowns. Qualitative identifications by a single UV wavelength for UV/Vis-generated chromatograms are possible with reference compounds, although not selective enough to differentiate structurally-similar organic molecular structures that respond to the monitoring wavelength or co-elute if not separated.

It is common in heritage science analysis to use qualitative analysis for identifications based on the relative retention times (rrt) of several peaks in the chromatogram peak of the unknown sample to the chromatographic “fingerprint” of known materials, which is helped by having a number of characteristic compounds in the sample. Leading laboratories in the heritage science field have hundreds of references in their analytical databases, built up over many years and reflecting research specialisms and interests. Much has been done since the 1990s to identify the characteristic compounds in the most important dyes of historical interest by HPLC-PDA, UHPLC-PDA, LC-PDA-ESI MS^n and LC-MS, and there are many published articles and conference proceedings (Wouters and Verhecken, 1989; Fereirra et al., 2001; Peggie et al, 2008; Serrano et al., 2015; Han, 2015; Mouri and Laursen, 2012; Liu, J. et al., 2013; Blackburn, 2017; Nowik 2001; Maarten et al., 2007; Kirby et al., 2017] and key reference books (Cardon, D., 2007; Hofenk de Graaff, 2014). *Dyes in History and Archaeology* has since 1981 been the leading international annual event for sharing

research between specialists, and its post-prints produced since 1986 cover the most important aspects of dyes from the past and their research. The Mass Spectrometry and Chromatography Group (MaSC) and the Museum of Fine Art Conservation & Art Materials Encyclopedia Online (CAMEO) have established shareable database, although analytical differences between laboratory equipment, analysts and consumables make searchable data comparisons challenging. Two major European Union-funded projects have developed and reviewed historical dye analysis methods and references materials – the Monitoring of Damage in Historic Tapestries[1] and CHARISMA[2] concluded that variations in methods and practice between labs did not adversely affect identifications.

Polymer chain lengths and molecular weights need references for accurate interpretation of results, but for natural materials like cellulose there is no one standard for polymer size and weight because it is a natural polymer with a distribution. So for SEC polystyrene is used even though this chemically different to cellulose and introduces errors into molar mass calculations and doesn't account for the skewing influences of natural compounds like hemicelluloses (Łojewski et al., 2011).

Qualitative analysis can be reliable for comparing and identifying diagnostic marker compounds even when structural compositions are not known, as is the case for a marker compound in the red brazilwood dye called Nowik A after the analyst who concluded its reliability as an identifier (Nowik, 2001). Statistical treatment of chromatographic and spectral data treatment, like principle component analysis (PCA), is enabling better correlation between historical materials and past production methods (Berbers, Tamburini, van Bommel, & Dyer, 2019).

All analytical methods for historical organic materials are challenged by chemical degradation from the environment. Light, humidity and temperature can induce oxidation, hydrolysis and photolysis reactions which affect organic compounds resulting in visual and physical changes to materials like yellowing, fading and embrittlement. These degradative effects change a material's molecular composition and hence affect key marker compounds and chemical profiles for chromatographic analysis. Although this sensitivity is problematic for identification, it makes chromatography an advantageous technique for conservation science investigations of degradation and preservation. Examples of chromatography-based degradation studies for library and archive collections include causes and effects for celluloid and acetate film cels (Richardson, Giachet, Schilling & Learner, 2014), Turkey red dyes in textile pattern books (Wertz, Quye, France, Tang & Richmond, 2017), iron gall inks on manuscripts (Centeno et al., 2016) and (Axelsson et al., 2016).

Case studies

Synthetic aniline dyes in dye books

In textile history studies, dyed textiles in books with textual context, dated authorship and preserved colour are as significant as provenanced textiles in museums (Sykas, 2005). The written text in historical dye books is highly informative about the material and practice of dyeing. However, what dyers valued most was the resulting colour (Cardon, 2016). These dyed textiles are a unique and often attractive feature of dyers' books, and present direct material evidence for historical dye colours that is visually engaging . As expected, historical dyers' books with textual information about dye materials and dyeing processes accompanied by coloured textile representations of the end results are of great value to anyone pursuing the history of textiles and dress.

Until the mid-19th century, dyes came from natural sources like plants, woods, insects, lichens and shellfish. The first synthetic dye appeared on the market in 1857 when aniline purple, the first so-

[1] see https://cordis.europa.eu/project/rcn/61585/factsheet/en (Accessed 10-11-2019)
[2] see https://cordis.europa.eu/project/rcn/92569/reporting/en (Accessed 10-11-2019)

called ‘coal tar colour‘, was commercially manufactured. From 1859, these new synthetic dyes were available in quantities that were economically viable for the dye industry to use. Books about commercial dyeing with these new dyes started being published for industry and the training of increasing numbers of dyers needed in the factories and mills. Some give textual information and some include dyed textile samples. The historical significance of these books have appealed to historians of science and technology (Travis, 1993), and to dress and fashion historians too. For this reason, the management of library collections of dyers’ pattern books is important for research, and why dye analysis has entered into library collection care and research (Wertz, Quye, France, Tang & Richmond, 2017).

As the significance and research value of library books with dyed textiles grows and new discoveries and connections between them and textile culture are made, so too does the need to confirm that the dyes are what the text says or implies that they are. This need for dye identification is important not only to authenticate the evidence, but also to predict risks of colour changes from exposure during study and exhibition. Dye receipts accompanying patterns in dye books normally name the dyes used. So why, then, is dye analysis of the patterns necessary for conservation and preservation? There are two reasons, concerning authenticity and preservation. The authenticity issue is that the material content of dye books has been little studied to date, so there is uncertainty whether the dyers or suppliers of the patterns always used the dyes given in the written receipt text. This is pertinent for published dye books where multiple copies were printed and patterns had to be entered into each one.

A heritage science research project called “Dye-Versity” was started in 2016 by the CTCTAH at the University of Glasgow. Over 200 patterns from twenty two books published between 1862 and 1893 on the early aniline triphenylamine dyes of commercial interest to the textile industry have been analysed so far by UHPLC-PDA, with confirmatory LC-ESI MS analysis by the Department of Chemistry and Industrial Chemistry at the University of Pisa (Quye et al., 2017). Consistent results are giving confidence that the named dyes for the patterns are indeed reliable, and the analysis of two patterns dyed with Hofmann violets in *Dyeing and Calico Printing* by Frederick Crace-Calvert, published in 1876, is a good example of the findings (Figure 4). Crace Calvert, an experienced and well-regarded dyer, describes the commercial synthesis of Hofmann violet as a reaction between rosaniline from magenta with methyl and ethyl iodides in the presence of alcohol (ethanol) and potassium or sodium hydrate. He gives two methods – one to make a red shade by reacting either ethyl iodide or methyl iodide with rosaniline, and the other to make a blue shade with a mixture of methyl iodide and ethyl iodide.

The analytical results from the patterns analysed so far for Dye-Versity show that with the chemistry of the dyes in the patterns matches the chemistry of the accompanying text (Figure 5), a remarkable feat given that these dyes were made before chromatography and spectroscopy were available to the dye manufacturers and chemists. Consequently, the significance of these unassuming books on library shelves is being raised so that many more researchers can access and use them (Kamposiori & Crossley, 2019).

Non-invasive analysis of VOCs

The proviso for chromatography in most cases is that sampling is needed. There are certain situations where the vapours from and around an aged or degraded material are distinctive enough that no sample needs taken from the object itself to identify the material or to make some judgement about its state of degradation. The distinctive smells of aged and degrading paper, leather and plastics are due to volatile organic compounds (VOCs) being released. Understandably this has intrigued conservators and scientists and led to research projects to capture VOCs from aged organic materials in objects and characterise the vapours by chromatographic analysis (Gibson et al,

2012; Curran et al., 2016). To do this, the airborne compounds need trapped and concentrated. The method to do this is called solid phase microextraction (SPME), which has been around since the late 1980s. SPME is one of the few instances of non-invasive sampling for chromatographic analysis in heritage applications. The air around the object that is releasing the VOCs is sampled by drawing it over a fibre of silica coated with a sorbent that attracts the compounds of interest. These compounds are trapped on the absorbent until they are released by being heated as part of the analysis, invariably by GC-MS. VOCs of furfural from degraded paper and aldehydes, and minor long-chain fatty acid from wood resins in ageing cotton/linen rag and wood pulp paper to be detected and studied in this way (Clark, Calvillo, Roosa, Green, & Ganske, 2011).

Although it sounds easy, SPME has tricky elements and there are a number of analytical considerations to be aware of. Firstly, the object needs enclosed in airtight chamber of glass or an impervious flexible plastic material that can form a perfect seal and does not release VOCs itself (a big ask of plastics or sealants) with a close-sealing, non-leaking port that the SPME fibre can be introduced into and removed from. VOC release from objects will depend on its surface area - a closed book compared to an open one – so this also needs factored in. Then the air needs to be stable in terms of temperature and %RH and in equilibrium with the object to ensure the sample is representative. The length of time of sampling is also critical, as is the chemistry and thickness of the sorbent coating on the silica fibre. The sorbent coating is selective and not all the compounds that the delicate human sense of smell can pick up will be attracted to the sorbent.

A promising new technique called Select Ion Flow Tube Mass Spectrometry (SIFT-MS) is pushing the boundaries of non-invasive qualitative trace organic analysis for heritage science (Le Nasa, Mattonai, Modugno, Degano & Ribechini, 2019). SIFT-MS is an ultra-soft chemical ionisation mass spectrometric technique with three advantages over SPME GC-MS: the air sample is drawn directly into the MS through a thin inert needle probe (0.8 mm outer diameter) with no effects from pre-deposition or pre-concentration so that all detectable molecules in the air sample can be studied; the equipment is portable and can be taken to the object; and the sampling time is quick, within 1 minute per object. SIFT-MS was trialled alongside py-GC MS and SPME-GC MS in a VOC study of modern comic books inside plastic bags - a mixture of organic inks and coatings on the paper substrate – to identify and quantify over 40 VOCs from the books alone (Figure 6). SIFT-MS detected and confirmed marker VOCs for furfural and isopropyl esters from aged cellulose in paper and photo-oxidised polystyrene from the printed finish that can be early warning signs for paper embrittlement and yellowing. With the rise of synthetic materials in modern printing and more plastics and synthetic dyes entering collections, non-invasive analytical techniques like SIFT-MS are important advancements for targeted and specific analytical surveys of large collections of contained artefacts in libraries, archives and museums.

References

Artioli, Gilberto. 2010. Scientific methods and cultural heritage: an introduction to the application of materials science to archaeometry and conservation science. Oxford: Oxford University Press. DOI:10.1093/acprof:oso/9780199548262.003.0003

Axelsson, K. M., Larsen, R., Sommer, D.V.P., & Melin, R. (2016). Degradation of collagen in parchment under the influence of heat-induced oxidation: Preliminary study of changes at macroscopic, microscopic, and molecular levels. *Studies in Conservation, 61*(1), 46-57. doi: 10.1179/2047058414Y.0000000140.

Banou, P., Alexopoulou, A., Chranioti, C., Tsimogiannis, D., Terlixi, A.-V., Zervos, S., & Singer, B. W. (2016). The effect of oil binders on paper supports via VOC analysis. *Journal of cultural heritage, 20*, 589-598.

Bartl, B., Trejbal, J., Ďurovič, M., Vašíčková, S., & Valterová, I. (2012). Analysis of efflorescence on surface of beeswax seals. *Journal of cultural heritage, 13*(3), 275-284.

Berbers, S. V., Tamburini, D., van Bommel, M. R., & Dyer, J. (2019). Historical formulations of lake pigments and dyes derived from lac: A study of compositional variability. *Dyes and Pigments, 170*, 107579.

Blackburn, R.S. (2017). Natural dyes in madder (Rubia spp.) and their extraction and analysis in historical textiles. *Coloration Technology 133*(6), 449-462. doi: 10.1111/cote.12308.

Bonaduce, I., Odlyha, M., Di Girolamo, F., Lopez-Aparicio, S., Grøntoft, T., & Colombini, M. P. (2013). The role of organic and inorganic indoor pollutants in museum environments in the degradation of dammar varnish. *Analyst, 138*(2), 487-500. doi: 10.1039/C2AN36259G

Breek, R., & Froentjes, W. (1975). Application of pyrolysis gas chromatography on some of Van Meegeren's Faked Vermeers and Pieter de Hooghs. *Studies in Conservation, 20*(4), 183-189.

British Standard Institution, European. 2012. Conservation of Cultural property - Methodology for sampling from materials of cultural property - General rules. BS EN 16085:2012. London: BSI

Cardon, D. (2007). *Natural dyes: sources, tradition, technology and science*. London: Archetype Publications.

Cardon, D. (2016). *The Dyers Handbook. Memoirs of an 18th Century Master Colourist*. Oxford and Philadelphia: Oxbow Books.

Centeno, S. A., Bronzato, M., Ropret, P., Zoleo, A., Venzo, A., Bogialli, S., & Badocco, D. (2016). Composition and spectroscopic properties of historic Cr logwood inks. *Journal of RAMAN spectroscopy, 47*(12), 1422-1428.

Clark, A. J., Calvillo, J. L., Roosa, M. S., Green, D. B., & Ganske, J. A. (2011). Degradation product emission from historic and modern books by headspace SPME/GC–MS: evaluation of lipid oxidation and cellulose hydrolysis. *Analytical and bioanalytical chemistry, 399*(10), 3589-3600.

Colombini, M. P., & Modugno, F. (2009). *Organic mass spectrometry in art and archaeology*: John Wiley & Sons.

Cooksey, C. J. (2017). Quirks of dye nomenclature. 7. Gentian violet and other violets. *Biotechnic & Histochemistry:*1-7. doi: 10.1080/10520295.2017.1286038.

Degano I., E. Ribechini , F. Modugno & M. P. Colombini (2009) Analytical Methods for the Characterization of Organic Dyes in Artworks and in Historical Textiles. *Applied Spectroscopy Reviews*, 44:5, 363-410, DOI:10.1080/05704920902937876

de la Rie, E. R. (1987). The influence of varnishes on the appearance of paintings. *Studies in Conservation, 32*(1), 1-13.

Dyer, J., Verri, G., & Cupitt, J. (2013). *Multispectral Imaging in Reflectance and Photo-induced Luminscence Modes: A User Manual*: British Museum.

Dyer, J., Tamburini, D., O'Connell, E.R.,, & Harrison, A. (2018). A multispectral imaging approach integrated into the study of Late Antique textiles from Egypt. *PLOS ONE 13*(10):e0204699. doi: 10.1371/journal.pone.0204699.

Ferreira, E.S.B., Heeren, R.M.A, van den Berg, K.J, Maines, C., Sutherland, K., & Higgitt, C. 2009. Mass Spectrometry of Art and Cultural Heritage. *International Journal of Mass Spectrometry 284*(1–3), 1-170.

Ferreira, E., Quye, A., McNab, H, Hulme, A.N., Wouters, J., & Boon, J. (2002). Development of analytical techniques for the study of natural yellow dyes in historic textiles. *Dyes in History & Archaeology* 16/17, 179 -186.

Ford, L., Rayner, C.M., & Blackburn, R.S. (2015). Isolation and extraction of ruberythric acid from Rubia tinctorum L. and crystal structure elucidation. *Phytochemistry 117*, 168-173. doi: https://doi.org/10.1016/j.phytochem.2015.06.015.

Germinario, G., Garrappa, S., D'Ambrosio, V., van der Werf, I. D., & Sabbatini, L. (2018). Chemical composition of felt-tip pen inks. *Analytical and bioanalytical chemistry, 410*(3), 1079-1094.

Gibson, L.T., Ewlad-Ahmed, A., Knight, B., Horie, V., Mitchell, G., & Robertson, C.J. (2012). Measurement of volatile organic compounds emitted in libraries and archives: an inferential indicator of paper decay? *Chemistry Central Journal 6,* 42. doi: 10.1186/1752-153x-6-42.

Granzotto, C., & Sutherland, K. (2017). Matrix assisted laser desorption ionization mass fingerprinting for identification of Acacia gum in microsamples from works of art. *Analytical chemistry, 89*(5), 3059-3068.

Hallett, K., & Howell, D. (2005). *Size exclusion chromatography as a tool for monitoring silk degradation in historic tapestries.* Paper presented at the Scientific analysis of ancient and historic textiles: informing preservation, display and interpretation: postprints, first annual conference, 13-15 July 2004.

Han, B., Daheur, G., & Sablier, M. (2016). Py-GCxGC/MS in cultural heritage studies: an illustration through analytical characterization of traditional East Asian handmade papers. *Journal of analytical and applied pyrolysis, 122*, 458-467.

Han, J. (2015). Botanical Provenance of Historical Chinese Dye Plants. *Economic Botany 69*(3), 230-239. doi: 10.1007/s12231-015-9314-y.

Hofenk de Graaff, J.H.T. (2004). *The colourful past : origins, chemistry and identification of natural dyestuffs*. Riggisberg, Switzerland; London: Abegg-Stiftung; Archetype.

Kamposiori, C., & Crossley, S. (2019). Evidencing the Impact and Value of Special Collections. *RLUK Report.* Research Libraries UK: London, 15-20

Kharbade, B., & Joshi, G. (1995). Thin-layer chromatographic and hydrolysis methods for the identification of plant gums in art objects. *Studies in Conservation, 40*(2), 93-102.

Kirby, J., van Bommel, M., & Verhecken, A. (2014). *Natural colorants for dyeing and lake pigments: practical recipes and their historical sources*. London: Archetype Publications Ltd.

Kirby, J., Cardon, D., Cooksey, C., Habib, V., Laursen, R., Quye, A., Schaeffer, T.T., Verhecken, A., & van Bommel, M.R. (2017). *The diversity of dyes in history and archaeology*. London: Archetype Publications Ltd.

Kogou, S., Neate, S. Coveney, C., Miles, A., Boocock, D., Burgio, L., Cheung, C.S., & Liang, H. (2016). The origins of the Selden map of China: scientific analysis of the painting materials and techniques using a holistic approach. *Heritage Science 4*(1), 1-24. doi: 10.1186/s40494-016-0098-x.

La Nasa, J., Mattonai, M., Modugno, F., Degano, I., & Ribechini, E. (2019). Comics' VOC-abulary: Study of the ageing of comic books in archival bags through VOCs profiling. *Polymer Degradation and Stability 161,*39-49. doi: 10.1016/j.polymdegradstab.2019.01.001.

Le Gac, A., Pessanha, S., Longelin, S., Guerra, M., Frade, J. C., Lourenço, F., . . . Carvalho, M. L. (2013). New development on materials and techniques used in the heraldic designs of illuminated Manueline foral charters by multi-analytical methods. *Applied Radiation and Isotopes, 82*, 242-257.

Liu, J., Mouri, C., Laursen, R., Zhao, F., Zhou, Y., & Li, W. (2013). Characterization of dyes in ancient textiles from Yingpan, Xinjiang. *Journal of Archaeological Science, 40*(12), 4444-4449.

Lluveras-Tenorio, A., Mazurek, J., Restivo, A., Colombini, M. P., & Bonaduce, I. (2012). Analysis of plant gums and saccharide materials in paint samples: comparison of GC-MS analytical procedures and databases. *Chemistry Central Journal, 6*(1), 115.

Łojewski, T., Zięba, K., Kołodziej, A., & Łojewska, J. (2011). Following cellulose depolymerization in paper: comparison of size exclusion chromatography techniques. *Cellulose, 18*(5), 1349.

Madariaga, J. M. (2015). Analytical chemistry in the field of cultural heritage. *Analytical methods, 7*(12), 4848-4876.

Masschelein-Kleiner, L. (1974). An improved method for the thin-layer chromatography of media in tempera paintings. *Studies in Conservation, 19*(4), 207-211.

Masschelein-Kleiner, L., Heylen, J., & Tricot-Marckx, F. (1968). Contribution à l'analyse des liants, adhésifs et vernis anciens. *Studies in Conservation, 13*(3), 105-121.

Mazurek, J., Laganà, A., Dion, V., Etyemez, S., Carta, C., & Schilling, M. R. (2019). Investigation of cellulose nitrate and cellulose acetate plastics in museum collections using ion chromatography and size exclusion chromatography. *Journal of cultural heritage, 35*, 263-270.

Mazzeo, R. (2017). *Analytical chemistry for cultural heritage*: Springer.

McNab, H., Ferreira, E. S., Hulme, A. N., & Quye, A. (2009). Negative ion ESI–MS analysis of natural yellow dye flavonoids—An isotopic labelling study. *International Journal of Mass Spectrometry, 284*(1-3), 57-65.

Mills, J., & Werner, A. (1952). Paper chromatography of natural resins. *Nature, 169*(4312), 1064.

Mouri, C., Aali, A., Zhang, X., & Laursen, R. (2014). Analysis of dyes in textiles from the Chehrabad salt mine in Iran. *Heritage Science, 2*(1), 20.

Nowik, W. (2001). The possibility of differentiation and identification of red and blue "soluble" dyewoods: determination of species used in dyeing and chemistry of their dyestuffs. *Dyes in history and archaeology 16/17*, 129 - 44.

Nowik. (2016). Dye analysis. In Cardon, D. 2016. *The Dyers Handbook. Memoirs of an 18th Century Master Colourist*. Oxford and Philadelphia: Oxbow Books. 148-149.

Odlyha, M. (2005). *Monitoring of damage to historic tapestries: the application of dynamic mechanical thermal analysis to model and historic tapestries.* Paper presented at the Scientific analysis of ancient and historic textiles: informing preservation, display and interpretation: postprints, first annual conference, 13-15 July 2004.

Ortega Saez, N. (2018). *Relation Between Historical Recipes and Current Preservation State of Black Dyed Wool: Recipe Based Research in North Western Europe, 1680-1850*. PhD thesis. University of Antwerp.

Peggie, D.A., Hulme, A.N., Mcnab, H., & Quye, A. (2008). Towards the identification of characteristic minor components from textiles dyed with weld (Reseda luteola L.) and those dyed with Mexican cochineal (Dactylopius coccus Costa). *Microchimica Acta 162*(3-4), 371-380. doi: DOI 10.1007/s00604-007-0866-0.

Poulin, J. (2007). Identification of indigo and its degradation products on a silk textile fragment using gas chromatography-mass spectrometry. *Journal of the Canadian Association for Conservation, 32*, 47-56.

Quye, A., Littlejohn, D., Pethrick, R. A., & Stewart, R. A. (2011). Investigation of inherent degradation in cellulose nitrate museum artefacts. *Polymer degradation and stability, 96*(7), 1369-1376.

Quye, A., Cheape, H., Burnett, J., Ferreira, E., Hulme, A., & McNab, H. (2003). An historical and analytical study of red, pink, green and yellow colours in quality 18th and early 19th century Scottish tartans. *Dyes in History & Archaeology* 19, 1-12.

Quye, A., Hallett, K., & Herrero, C. (eds.). (2009). *Wroughte in gold and silk: preserving the art of historic tapestries*. Edinburgh: NMS Enterprise Ltd, Publishing.

Quye, A., Littlejohn, D., Pethrick, R.A., & Stewart, R.A. (2011). Investigation of inherent degradation in cellulose nitrate museum artefacts. *Polymer Degradation and Stability 96(7),* 1369-1376. doi: 10.1016/j.polymdegradstab.2011.03.009.

Quye, A., & Strlič, M. (2019). *Icon Heritage Science Group Ethical Sampling Guidance*. London: Institute of Conservation. ISBN 978-1-5272-3826-8

Quye, A., Wertz, J. & Degano, I. (2017). Dye analysis of textile patterns for aniline colours in nineteenth century British dyeing manuals by UHPLC-PDA and LC-ESI MS. *36th Annual Meeting of Dyes in History and Archaeology (DHA36)*, London, UK, 26-28 Oct 2017.

Richardson, E., Giachet, M.T., Schilling, M., & Learner, T. (2014). Assessing the physical stability of archival cellulose acetate films by monitoring plasticizer loss. *Polymer Degradation and Stability* 107, 231-236. doi: ttps://doi.org/10.1016/j.polymdegradstab.2013.12.001.

Rushworth, I. D., Higgitt, C., Smith, M., & Gibson, L. T. (2014). Non-invasive multiresidue screening methods for the determination of pesticides in heritage collections. *Heritage Science, 2*(1), 3. doi: 10.1186/2050-7445-2-3

Saliu, F., Modugno, F., Orlandi, M., & Colombini, M. P. (2011). HPLC–APCI-MS analysis of triacylglycerols (TAGs) in historical pharmaceutical ointments from the eighteenth century. *Analytical and bioanalytical chemistry, 401*(6), 1785-1800.

Serrano, A., van Bommel, M.R., & Hallett, J. (2013). Evaluation between ultrahigh pressure liquid chromatography and high performance liquid chromatography analytical methods for charactiersing natural dyestuffs. *Journal of Chromatography A 1318*, 102-11. doi: 10.1016/j.chroma.2013.09.062.

Serrano, A., van den Doel, A. van Bommel, M.R., Hallett, J., Joosten, I., & van den Berg, K.J. (2015). Investigation of crimson-dyed fibres for a new approach on the characterization of cochineal and kermes dyes in historical textiles. *Analytica Chimica Acta* 897, 116-127. doi: http://dx.doi.org/10.1016/j.aca.2015.09.046.

Shahid, M., Wertz, J., Degano, I., Aceto, M., Khan, M.I., & Quye, A. (2019). Analytical methods for determination of anthraquinone dyes in historical textiles: A review. *Analytica Chimica Acta* 1083:58-87. doi: 10.1016/j.aca.2019.07.009.

Shedrinsky, A. M., Grimaldi, D. A., Boon, J. J., & Baer, N. S. (1993). Application of pyrolysis-gas chromatography and pyrolysis-gas chromatography/mass spectrometry to the unmasking of amber forgeries. *Journal of analytical and applied pyrolysis, 25*, 77-95.

Skytte, L., Rasmussen, K. L., Svensmark, B., Ryhl-Svendsen, M., & Brimblecombe, P. (2017). Monitoring the accumulated water soluble airborne compounds deposited on surfaces of showcases and walls in museums, archives and historical buildings. *Heritage Science, 5*(1), 1. doi: 10.1186/s40494-016-0115-0

Striegel, M. F., & Hill, J. (1997). *Thin-layer chromatography for binding media analysis*: Getty Publications.

Stuart, B. (2007). *Analytical techniques in materials conservation*. Chichester, England;Hoboken, NJ;: John Wiley & Sons.

Sykas, P.A. (2005). *The Secret Life of Textiles: Six Pattern Book Archives in North West England*. Bolton Museum, Art Gallery & Aquarium: Bolton.

Taylor, G.W. (1992). The chemistry of madder dyeings: application of semi-quantitative TLC. *Dyes in History and Archaeology postprints of the 10th annual meeting of Dyes in History and Archaeology*, National Gallery London 1991

Travis, A.S. (1993). *The rainbow makers : the origins of the synthetic dyestuffs industry in western Europe*. London: Associated University Presses.

Troalen, L.G., Phillips, A.S., Peggie, D.A., Barran, P.E., & Hulme, A.N. (2014). Historical textile dyeing with Genista tinctoria L.: a comprehensive study by UPLC-MS/MS analysis. *Analytical Methods 6*, 8915-8923. DOI: 10.1039/C4AY01509F (

van Bommel, M. R., Berghe, I. V., Wallert, A. M., Boitelle, R., & Wouters, J. (2007). High-performance liquid chromatography and non-destructive three-dimensional fluorescence analysis of early synthetic dyes. *Journal of Chromatography A, 1157*(1-2), 260-272.

van der Werf, I. D., Calvano, C. D., Germinario, G., Cataldi, T. R., & Sabbatini, L. (2017). Chemical characterization of medieval illuminated parchment scrolls. *Microchemical Journal, 134*, 146-153.

Wagner, B., Bulska, E., & Sobucki, W. (2008). Magnesium distribution in paper subjected to deacidification investigated by means of laser ablation inductively coupled plasma mass spectroscopy. *Journal of cultural heritage, 9*(1), 60-65.

Walton, P., & Taylor, G. (1991). The characterisation of dyes in textiles from archaeological excavations. *Chromatography and analysis*, 5-7.

Wouters, J. (1985). High performance liquid chromatography of anthraquinones: analysis of plant and insect extracts and dyed textiles. *Studies in Conservation, 30*(3), 119-128.

Wouters, J., & Verhecken, A. (1989). The coccid insect dyes: HPLC and computerized diode-array analysis of dyed yarns. *Studies in Conservation 34*, 189 - 200.

Zhang, X., & Laursen, R. A. (2005). Development of mild extraction methods for the analysis of natural dyes in textiles of historical interest using LC-diode array detector-MS. *Analytical chemistry, 77*(7), 2022-2025.