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# Gas Chromatography and Analysis of Binding Media of Museum Objects: A Historical Perspective

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**Abstract.** This contribution covers the major historic milestones of the evolution of gas chromatography (GC) from its beginnings to its current status as one of the most powerful analytical separation techniques, and demonstrates simultaneously how this technique has enabled and continuously improved the analysis of organic binding media in objects of cultural heritage. After an introduction into the basics of chromatography, the development of GC is traced from its emergence in the late 1800s as a mere preparative technique through a period of relative stagnation into the mid of the 20th century. Then, the 1950s are covered by highlighting the major advances in theory and technology within this decade, all of which contributed to firmly consolidate the status of GC as a modern analytical separation technique. From there the maturing of GC is followed through the 1960s up to the present days, a period being marked by the transition from packed to capillary columns; the essential adaptation of injection and detection devices; the replacement of glass by fused silica as column material; major progresses in stationary phase chemistry; and, finally, the advent of the hyphenation of GC with mass spectrometric detection devices. Throughout this survey, examples of applications of contemporary GC techniques to binding media analysis are discussed to provide an illustrative historic record of the continuous improvements achieved. The account will be closed with critical reflections on GC's current relevance to and future role in the analysis of binding media in objects of cultural heritage.

**Keywords**. Natural organic binding media, gas chromatography, history, cultural heritage, museum objects.



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## 1. INTRODUCTION

Knowledge of both the genuine techniques and materials employed in the creation of objects of cultural heritage are of crucial importance for their scientific and artistic analysis. Objects of cultural heritage (for which we shall use in the following the term "museum objects") can consist of nearly innumerably inorganic and organic materials. Nowadays, the reliable identification of the chemical nature and source of these materials is of eminent importance to guide the development of object-appro-

priate conservation and restoration techniques. In addition, knowledge on the constituting materials in museum objects may provide a host of other important scientific insights, such as a historic record of the social and economic conditions at the time the respective objects were fabricated, and information on the contemporary status of craftsmanship, and intercultural exchange and technology transfer. Elucidation of the geographic provenience of materials integrated in museum objects may also help to trace both ancient trade relationships and trade routes. And most importantly, a detailed profile of the constituting materials in museum objects may provide valuable evidence concerning the period of production and geographic origin, and thus for reliable establishment of authenticity of a given object of cultural heritage.

The present contribution will focus on one class of these materials, *viz.* natural organic binding media (in the following termed binding media or binders).[1, 2] The determination of these materials in museum objects has a long tradition, and many analytical approaches have been applied to this purpose. These methods range from visual examination over microchemical tests to the current state-of-the-art spectrometric and separation methods, such as liquid and gas chromatography, often used in conjunction with powerful mass-sensitive detection devices. Gas chromatography (GC), probably the most frequently employed analytical technique for the identification of binding media in museum objects, will be the central subject of this contribution. Specifically, the intent of this account is twofold: our first objective is to provide an overview on the historical development of GC from its humble beginnings to its current mature status, and to pay credit to those scientists who through their ingenious contributions have advanced GC to one of the most powerful of the current analytical separation techniques. In addition, and as our second goal, we wish to demonstrate how the continuous technological advances achieved in GC methodology over the last five decades have made possible addressing the formidable challenges associated with binding media analysis in museum objects.

Before advancing to the discussion of the historic milestones in the synergistic evolution of GC methodology and concurrent improvements in binding media analysis, we find it beneficial to provide a brief section clarifying some fundamental terminology and aspects of chromatography.

## 2. METHODOLOGY OF CHROMATOGRAPHY

A chromatographic system consists of two immiscible phases. Under operational conditions, one phase (the stationary phase; either a solid or a liquid) is kept immobile whereas the second phase (the mobile phase; either a gas or a liquid) is forced to flow continuously through the separation system. After introduction of the sample into the system, the contained analytes are distributed between the mobile and the stationary phase according to their relative affinities, leading to their physical separation.

Chromatographic methods can be categorized by several criteria, e.g. (1) according to the geometry of the system; (2) the physical state of the phases and the operative interphase distribution mechanisms; and (3) by the mode of sample introduction.

Concerning geometry, a chromatographic system may exhibit a (quasi-)two-dimensional format, such as in thin layer and paper chromatography; however, we will not discuss these methods in the following. In contrast, in column chromatography, the stationary phase is situated within a tube, generally referred to as column, through which the flow of the mobile phase is directed.

With regard to the physical state of the phases, chromatographic methods are classified either as gas chromatography (GC) or as liquid chromatography (LC), depending on the state of the mobile phase. GC and LC may further be categorized based on the physical state of the employed stationary phase as gas solid (GSC) and gas liquid (GLC), and as liquid liquid (LLC) and liquid solid (LSC) chromatography. Note that in the present contribution the method is termed *partition* chromatography in case that the analytes are distributed between a liquid or a gaseous mobile phase, respectively, and a liquid stationary phase; distribution is based on *absorption* in the two phases. In *adsorption* chromatography, in contrast, the stationary phase is a solid surface (e.g. as in ion exchange chromatography).

The mode of sample introduction may involve either the injection of a sample amount being small relative to the volume of the system, or, alternatively the continuous introduction of sample solution. For the former mode - the *elution* mode - the sample is introduced as a narrow plug at the inlet of the column into the flowing mobile phase. During the transport of the sample through the column, the contained analytes are separated into individual sample zones with the mobile phase in between. It should be mentioned that for almost all analytical applications, elution mode chromatography is employed, and the term chromatography (that will be often used in the present contribution) is currently a common synonym of elution mode column partition chromatography.

In the latter mode - the *frontal analysis* mode - the sample is either continuously fed as the mobile phase

into the column, or may be continuously introduced dissolved in the mobile phase. In this mode only the first eluting zone contains pure analyte, with the subsequently emerging zones containing mixture of analytes, the composition of which being determined by the relative affinity of the analytes towards the stationary phase. This mode of chromatography is normally not used for analytical, but rather for preparative purposes. It shall be mentioned that other modes of chromatography do exist, such as displacement chromatography or chromathermography, but these are irrelevant for the current topic and therefore are not further discussed here.

Elution mode GLC is currently the sole gas chromatographic method employed for the analysis of binding media in museum objects. Historically, GLC has emerged from a number of precursor techniques, which will be outlined in some detail in the following account on the invention and evolution of chromatographic techniques relevant to binding media analysis. However, prior to these discussions, a brief overview of the most important classes of organic compounds used as binding media shall be given.

## 3. NATURAL ORGANIC BINDING MEDIA IN OBJECTS OF THE CULTURAL HERITAGE

Remarkably, despite the fact that Nature provides a sheer unlimited repertoire of organic compounds, only a few classes have been applied as binding media in museum objects. In accordance with their chemical nature, binding media encountered in art objects can be categorized into six classes (see e.g. refs. [2, 3]), *viz*. waxes, resins, oils and fats, animal glues, plant gums, and bituminous material. Representative compounds for each of these classes are depicted in Figure 1.

The main constituents of natural waxes are long chain *n*-alkanes (and of beeswax also esters of long-chain fatty acids and alcohols, and smaller amounts of free fatty acids). Waxes are found in museum objects amongst others as varnishes and coatings, or as matrix components of wax models, and have been widely used in antiquity as binders for pigments in Encaustic painting.

Natural resins are products secreted from woody plants, mainly consisting of complex mixtures of diterpenoids or triterpenoids (compounds with either 20 or 30 C-atoms in their molecules), preferentially of cyclic structures; these compounds often contain C=C double bonds, and bear hydroxy and carboxylic groups. It is worth noting that a given plant produces either diterpenoid or triterpenoid resins, but not both. In museum objects, resins have been generally employed as varnishes, for coatings, additives, and consolidants.

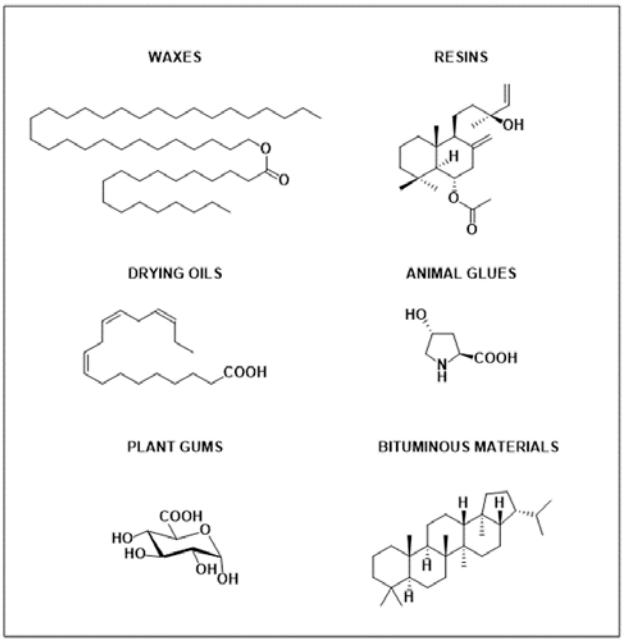


**Figure 1.** Representative constituents of natural organic binding media. (i) Waxes: Triacontanyl palmitate (in bees wax). (ii) Diterpenoic resins: Larixyl acetate (in Venetian turpentine). (iii) Drying oils: Linolenic acid (9,12,15 octadecatrienoic acid (in linseed oil). (iv) Animal glues: Hydroxyproline (in collagens). (v) Plant gums: Glucuronic acid (in gum Arabic). (vi) Bituminous materials: Hopane in asphaltenes.

Chemically, oils and fats are triglycerides of long-chain fatty acids. Drying oils, employed as binders of the pigments for oil paintings in Western art, contain a high proportion of unsaturated fatty acids. The commonly used linseed oil mainly consists of the C18 fatty acids oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids (the suffix 18:2 denotes the presence of two C=C double bonds in a fatty acid containing 18 C-atoms). Linoleic and linolenic acids possess isolated double bonds, in contrast to eleostearic acid, a $C_{18:3}$ acid with three conjugated C=C double bonds, a main constituent of tung oil, which was applied in some objects as substitute for linseed oil in the first half of the 20$^{th}$ century.[4, 5] The drying process is a radical-induced oxidative polymerization of the unsaturated fatty acids, leading to the formation of a three-dimensional cross-linked network. This process is accompanied by cleavage of the double bond and formation of short chain $C_7$, $C_8$, $C_9$ dicarboxylic acids, which are generally detectable in aged dried oil.

Egg, casein and collagens are the main animal glues used; they are proteins and consist of peptide chains containing essentially all of the twenty natural amino acids. However, the amino acid hydroxyproline is a specific constituent of collagens, formed by post transla-

tional modification of proline, and is a unique marker of this type of glue. Egg was widely used as a binder of pigments in tempera painting, the dominating painting technique used prior to the invention of oil painting in Western art in the early 15th century.

Plant gums are polysaccharides, composed of a range of monosaccharides and uronic acids (typically glucuronic and galacturonic acids). Frequently employed plant gums in binding media are gum Arabic, gum tragacanth and cherry gum. Occasionally, starch has also been applied.

Bituminous material is a very complex mixture of high molecular mass compounds, and is a generic term for two classes of substances, amongst bitumen and asphalt are natural materials, and tars and pitches are technical products. Pitches are directly resulting from pyrolysis of wood or resin, while tars represent the products of a subsequent distillation. However, these compounds are rarely encountered as binders in museum objects, and will not be treated here in more detail. Readers interested in an in-depth treatise on organic binding media and coatings are directed to ref. [2].

As an essential prerequisite, GC analysis requires the analytes of interest (except for pyrolysis GC, see below) to be sufficiently volatile and thermally stable to avoid decomposition at the typically employed elevated temperatures. Therefore, GC is not directly applicable to the majority of the common binding media. However, this limitation can be conveniently overcome by chemical transformation of these materials into more volatile compounds, e.g. by de-polymerization via acid-catalyzed hydrolysis and appropriate derivatization of the emerging low-molecular mass constituents.

## 4. A BRIEF REVIEW OF THE GENERAL PRINCIPLES AND THE TERMINOLOGY OF CHROMATOGRAPHY

Two main parameters determine the separability of analytes in chromatography, namely the *retention factor*[1] and the *plate number*[2]. The former parameter reflects the velocity by which a given analyte zone moves through the column, while the latter is a measure of the continuous broadening the zone underlies upon migration through the chromatographic system. Certainly, a combined knowledge of the properties of the chromatographic system and the analytes, and their interactions is key for an informed selection of favorable operational variables and parameters. While we wish to restrain from a detailed treatment of chromatographic theory, we consider it beneficial to familiarize interested readers with some fundamental relationships. We hope these will aid the understanding of crucial milestones that marked the development of GC from its beginning as a preparative technique to its present status as one of the most powerful methods for trace analysis.

### *4.1 Zone Migration: Retention Time, Retention Factor and Separation Selectivity*

The migration velocity, $u_i$, of a given analyte, $i$, through a chromatographic column with the mobile phase flow velocity, $v$, is determined by the analyte′s degree of distribution between the stationary (denoted by subscript $s$) and mobile phase (denoted by subscript $m$). In the steady state, the fraction of the analyte in the mobile phase is equal to the ratio of mole number $n_{i,m}$ to the total mole number $(n_{i,m}+n_{i,s})$ being $n_{i,m}/(n_{i,m}+n_{i,s})$, which can be also expressed as $1/[1+(n_{i,s}/n_{i,m})]$; the ratio $n_{i,s}/n_{i,m}$ is the mass distribution coefficient. In *partition* chromatography like GLC, the respective mole numbers are equal to $n = c.V$, the product of corresponding concentrations, $c$, and volumes, $V$. The analyte concentration ratio[3] between stationary and mobile phase is the partition coefficient, $K_i=c_{i,s}/c_{i,m}$, and the ratio of the volumes of stationary and mobile phase is named phase ratio, $q=V^s/V^m$. Combining these expression allows formulation of the fraction of the analyte in the mobile phase by

$$n_{i,m}/(n_{i,m}+n_{i,s})=1/(1+K_iq)=1/(1+ k_i) \quad (1)$$

We define $k_i$, the *retention factor,* as $k_i=K_iq$; it is identical with the mass distribution coefficient, and is one of the most important parameters for the description of any chromatographic process.

Since the fraction $1/(1+k_i)$ of the analyte in the mobile phase (see Equation 1) migrates with velocity $v$, and assuming that the rate of the mass exchange of the analyte between the two phases by distribution is fast, it follows that the entire analyte zone moves through the column with velocity $u_i$, which can be expressed as given in Equation 2a by

[1] We prefer to use the more explicatory term *retention factor* rather than *mass distribution ratio*, as proposed by IUPAC, or *capacity factor*, the more common term in older literature.

[2] Initially, in the classical literature of zone dispersion (see Chapters 4.2. and 5.5) the term *plate number* is named "*number of theoretical plates*".

[3] Note that in *partition* chromatography the concentrations in both phases are defined by moles per volume. In *adsorption* chromatography, in contrast, the concentration of the analyte at the stationary solid surface with area $A$ is given by moles per area [mol/$A$]. In this case the distribution coefficient $K$ is not dimensionless, but has the dimension of a length.

$$\begin{aligned} &a)\quad u_i = v/(1+k_i) = v/(1+K_i q) \\ &b)\quad t_{Ri} = L/u_i = (L/v)(1+k_i) = t_{R0}(1+k_i) \\ &c)\quad k_i = (t_{Ri} - t_{R0})/t_{R0} \end{aligned} \tag{2}$$

From Equation 2a it can be concluded that in GLC the migration velocity, $u_i$, of analyte, *i*, depends on three parameters, namely (i) on the mobile phase flow velocity *v*, and (ii) on the phase ratio $q=V^s/V^m$, with both of these parameters being equal, *i.e.* unspecific, for all analytes; and (iii) on the partition coefficient, $K_i$. This partition coefficient is an analyte-specific quantity, reflecting the distinct interactions a given analyte undergoes with the stationary and mobile phase, respectively. Differences in *K*, or more specifically in the retention factors *k* are mandatory to achieve analyte separation. The degree of separation depends on the ratio of the retention factors, the so-called *selectivity coefficient* $r_{ji}=k_j/k_i$ (with $k_j \geq k_i$), which is a measure for the *separation selectivity* of the system for a given pair of analytes, *i* and *j*.

For column chromatography, the retention or residence time, $t_{Ri}$, is the time the analyte needs to migrate with its velocity $u_i$ through the column with length, *L*; it is given by Equation 2b. The void or dead time, $t_{Ro}$ is the time the mobile phase requires to flow through the column over length, *L*. Experimentally, retention times are measured at the maximum of corresponding analyte concentration profiles (usually Gaussian) upon elution from the columns. Note that the retention time $t_{Ri}$ of the analyte depends on the same parameters as the migration velocity, *viz.* on the unspecific mobile phase velocity (and the column length), and on the analyte-specific retention factor, $k_i$ again emphasizing the importance of this parameter. The retention factor $k_i$ can simply be calculated from the measured retention time $t_{Ri}$ and the dead time $t_{Ro}$ according to Equation 2c.

### *4.2 Zone Broadening: Plate Height, Plate Number and Separation Efficiency*

It is important to recognize that different migration velocities of a pair of analytes in the chromatographic column are an essential but not a sufficient criterion for their successful separation. This is caused by the fact that the migrating analytes are continuously diluted by the mobile phase, *i.e.* they become dispersed within a larger volume and, as a consequence, their zones become broader; thus, neighboring zones of a given pair of analytes might overlap even if they possess different retention factors.

Under the premise that the chromatographic column is operated at constant temperature and the sample is introduced as an (infinitely) narrow plug into the column, the recorded concentration distribution of the analyte forms the typical Gaussian curve (usually referred to as a peak) due to various dispersion processes. The width of the peak is expressed by its standard deviation, σ. During migration, the peak variance, $\sigma_x^2$ in the length scale increases directly proportional to the migration distance, *x*, according to $\sigma_x^2 = H\,x$ The proportionally factor, *H*, is a characteristic parameter for the dispersion property of the chromatographic column, *i.e.* for its *efficiency*. For historical reasons, *H* is referred to as *height equivalent of a theoretical plate, or (theoretical) plate height* and has the dimension of a length. Note that the efficiency of a column must not be confused with its ability to separate compounds, efficiency (expressed by a figure) is a property that is strictly related to zone broadening.

In column chromatographic practice, however, the zone width is not measured in the length domain at a certain time (as it is done in planar chromatography). Rather, all components are permitted to traverse the entire column length *L* and are registered at the outlet of the column as a function of time. For convenience, the resulting peak widths are measured in the time domain, e.g. by the time-based standard deviation, $\sigma_{t,i}$. Both standard deviations, $\sigma_{t,i}$ and $\sigma_{x,i}$ (since at $x=L$, $\sigma_{x,i}$ can be written as $\sigma_{L,i}$), are related to the migration velocity, $u_i$, which is $L/t_{R,i}$ by

$$\begin{aligned} &\sigma_{L,i} = H\,L \\ &b)\ \sigma_{t,i}^2 = t_{R,i}^2 \times H/L \end{aligned} \tag{3}$$

The ratio $L/H=N$ is referred to as *the number of (theoretical) plates or (theoretical) plate number*, *N*, and is a measure for the *efficiency* of a given column with length *L* and plate height, *H*. The plate number, *N*, can be conveniently calculated from the time-based peak width and the corresponding retention time according to Equation 4a as

$$\begin{aligned} &a)\ N = t_{R,i}^2/\sigma_{t,i}^2 \\ &b)\ \sigma_{t,i} = t_{R,i}/\sqrt{N} \end{aligned} \tag{4}$$

From the rearranged Equation 4b it can be seen that $\sigma_{t,i}$ increases directly proportional to the retention time $t_{R,i}$ (the causes for this increase will be discussed in more detail in Chapters 5.5 and 7).

*4.3 The Chromatographic Resolution: the Quantity for the Degree of Separation*

As outlined above, the separation of a given pair of analytes, *i* and *j*, is governed by two processes, namely *zone migration* and *dispersion*. Certainly, there is a need for a quantity that expresses the degree of separation in a well-defined fashion considering the combined effect of these processes. This quantity is the *chromatographic resolution*, $R_{j,i}$.

It is obvious that for successful separation of a pair of analytes their retention times must differ (which means that the selectivity coefficient $r_{ji}$ must be larger than unity). It is, however, not meaningful to measure this difference ($t_{r,j}$–$t_{r,i}$) in absolute time units, because at a given retention time difference narrow peaks may be well resolved, while broad peaks may still strongly overlap. Therefore ($t_{r,j}$–$t_{r,i}$) is related to the width of the two peaks, given by their time-based standard deviations, $\sigma_{t,i}$ and $\sigma_{t,j}$. According to IUPAC, the degree of separation, the chromatographic resolution, is defined for this pair of analytes by

$$R_{j,i} = \frac{\left(t_{r,j} - t_{r,i}\right)}{2\sigma_{t,i} + 2\sigma_{t,j}} \tag{5}$$

The resolution as defined in Equation 5 is a dimensionless number; baseline separation of two peaks (of equal size) is obtained when the resolution has a value equal to or larger than 1.5.

However, Equation 5 is not very practical when resolution needs to be expressed as a function of variations in experimental parameters. Transforming this relationship considering Equations 2b and 4b provides a practically more useful expression for the chromatographic resolution being a function of retention factors and plate number [4] by

$$R_{ji} = \frac{1}{4}\frac{\left(k_j - k_i\right)}{k_i}\frac{k_i}{\left(1+k_i\right)}\sqrt{N} = \frac{1}{4}\left(r_{ji} - 1\right)\frac{k_i}{\left(1+k_i\right)}\sqrt{N} \tag{6}$$

From Equation 6 it is evident that the achievable chromatographic resolution is impacted by three terms (we chose to ignore the factor ¼): The middle term, the retention or retardation term, plays a minor role; it is relevant only to analytes with very small retention (e.g., very volatile compounds in thin film capillaries, see below). (ii) The third term, the efficiency term, contains the plate number, expressing the effect of peak broadening on resolution. Since separation depends on the square root of *N*, realizing a twofold improvement in resolution requires a fourfold increase in plate number. Practically, on the one hand, this could be achieved by employing a fourfold longer column, yet improvements in resolution would come at the prize of a fourfold increase in analysis time. On the other hand, efficiency can be enhanced by using columns with lower plate heights; this approach became feasible through the invention of open tubular capillary columns (see Chapter 7). Very significant improvements in resolution can be realized by changing separation selectivity, which is represented in the first term of Equation 6 by the selectivity coefficient, $r_{ji}$. Evidently, any variation in stationary phase and/or the operation conditions that results even in a minute increase of $r_{ji}$ will produce pronounced changes in resolution.

## 5. A SHORT HISTORY OF GAS CHROMATOGRAPHY

Decades before gas liquid chromatography was invented, separations were already carried out by liquid chromatography. The invention of liquid chromatography is attributed to M. S. Tswett, yet there were several earlier studies by other scientists applying essentially liquid solid chromatographic techniques in the frontal analysis mode for preparative purposes; e.g. in 1893, L. Reed separated salts by applying their solution onto a tube filled with kaolin as adsorbent[6]; and D.T. Day published first investigations with LSC in 1897 aiming at the separation of colored constituents characteristic for oils from different sources.[7]

*5.1 The Invention of Chromatography: Liquid Solid Chromatography*

In his first publication from 1903 Tswett, a Russian botanist, described the successful separation of plant pigments.[8] In his experiments, he applied a chlorophyll extract in ligroin (*i.e.* petroleum ether) at the top of a vertically arranged cylindrical glass tube (see Figure 2) filled with particles of a solid material with adsorptive abilities, and continued applying fresh ligroin. Tswett observed the formation of separated colored rings, which migrated through the tube and broadened during their migration.

Tswett coined for this separation technique the term "chromatographic method", first mentioned in 1906in

[4] Variation of the experimental conditions is needed only for a pair of closely migrating analyte, *i.e.* for analytes with very similar retention. In this case $k_j \approx k_i$ and $N_j \approx N_i = N$ However, note that even if the difference ($k_j$–$k_i$) is close to zero, the ratio $r_{ji}$ can be significantly larger than unit.

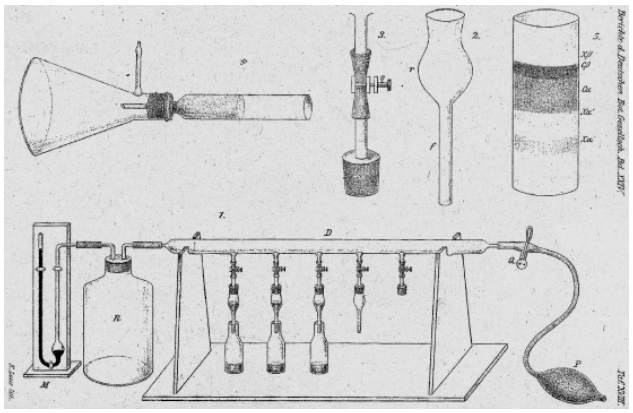

**Figure 2.** Tswett's device with four packed chromatographic glass columns. Drawing (1.): three columns filled with adsorbents for the separation of plant pigments. The columns had an inner diameter of 2-3 mm, and a length of 2-3 cm. Drawing (5.): Separated zones of 5 colored plant pigments (Chlorophylls and Xanthophylls) in a chromatographic packed column. From ref. [9] with permission.

two publications in a German journal.[9, 10] Interestingly, the name of this method persisted until nowadays (although it is a misnomer, as analytes processed by modern chromatography are not necessarily colored).

After a dormant period, Tswett´s ideas were revisited in 1931 by the biochemist R. Kuhn and his coworkers, who successfully used LSC to accomplish the separation of carotins and xantophylls.[11-14] In 1937, three decades after Tswett's reports on the chromatographic method, the first monograph dedicated to chromatography was published.[15] However, due to the limited scope of compound classes that could be addressed, the use of LSC remained somewhat limited in the scientific community. The ultimate breakthrough of liquid chromatography as a powerful separation technique came with the recognition that replacing solid by (solid-supported) liquid stationary phases allowed for an enormous extension and significant improvements of selectivity profiles. Certainly, this notion marked the birth of modern liquid liquid partition chromatography (LLC).

### *5.2 Liquid Liquid Chromatography*

The innovative concept of liquid liquid partition chromatography was published by Martin and Synge in 1941, including a model to express the efficiency of a column[16]; in the same journal issue the separation of *N*-acetylated amino acids in a column with water as liquid stationary phase (absorbed in silica gel) and chloroform with 0.5 to 1.0% *n*-butanol as mobile phase was described.[17] In appreciation of their pioneering work, J.P. Martin and R.L.M. Synge were jointly awarded the Nobel Prize in Chemistry 1952 "*… for their invention of partition chromatography*".

Chromatography employing liquid stationary phases allowed exploiting a much greater variety of solute interactions for tuning selectivity than LSC, and ultimately promoted LLC to one of the most important contemporary analytical separation methods. The practical utility of LLC was further potentiated by reducing the particle sizes of the packed bed down to the micrometer range and thus producing columns providing vastly enhanced column efficiencies. From this effort, *High Performance Liquid Chromatography* (HPLC) emerged, a chromatographic technique that from a general viewpoint is superior to GC for two reasons: One crucial limitation of GC is that its applicability is restricted to the relatively low number of sufficiently volatile and thermostable compounds, requirements that are certainly irrelevant to LLC. The second aspect that favors LLC over GLC is that in the latter technique selectivity emerges through interaction with the liquid stationary phase only, as the mobile phase is an inert gas. In contrast, in LLC, the mobile liquid phase offers an additional and highly versatile tool for varying separation selectivity via specific solvent-solute interactions. Taken together, these advantages enormously widen the general separation ability of LLC as compared to GLC, and explain the outstanding success of HPLC in modern analytical sciences.

### *5.3 Early GC: Gas Solid Chromatography*

Even at times predating Tswett´s introduction of LSC, the adsorption of gases or liquids on solid surfaces was actively investigated, with the first reports emerging at the beginning of the 19$^{th}$ century. In the early 20$^{th}$ century research was primarily devoted to the adsorption of gases on solid sorbents pursuing preparative applications. Specific areas of interest were, e.g. the purification or recovery of constituents of vapors, or the improvement of the effectivity of gas masks. Essentially all of these investigations were carried out by frontal analysis mode.[18-20]

In 1930s P. Schuftan introduced separation techniques for which he coined the general term "adsorption analysis", and which may well have been the first successful demonstration of gas solid chromatography for analytical purposes, but still at a micro-preparative scale. He applied this method for gas analysis in the technical area, and separated and quantified gases such as low-boiling hydrocarbons, carbon monoxide and hydrogen.

In early 1930s, Tswett's introduction of *elution* mode LSC returned from oblivion and was gradually adopted for GSC.[14] G. Hesse et al. described GSC separation using a carrier gas as mobile phase rather than the sample mixture as in frontal analysis. Remarkably, in 1943 the first separation by gas *liquid* chromatography appears to have been carried out by G. Damköhler and H. Theile.[21] Specifically, they achieved separations of methanol from ethanol, and benzene from cyclohexane, employing tubes filled with grained fired clay as solid support and glycerol as stationary liquid phase (it should be mentioned that their primary intention for the addition of glycerol was to deactivate the solid surface) and hydrogen or nitrogen as mobile phase. Unfortunately, their contribution, after having been published in a less renowned journal, did not find any resonance in the scientific community. In addition, as both authors were staff scientists employed at an institution [5] devoted to support the German war effort, their interest may have been redirected to issues more pressing than further research into this method.

The decisive step towards establishing GSC as a useful microscale separation technique was taken by E. Cremer and her coworkers, who constructed the first fully operational analytical gas chromatograph operating in elution mode, including a sufficiently sensitive homemade thermal conductivity cell as a detector. Interestingly, Cremer described the results in a manuscript that was accepted for publication in 1944, but which failed to appear in print due to the chaotic conditions at the end of World War II. Cremer's results were published years later, between 1949 and 1952[22-24], and even then they remained largely ignored. Anyhow, the application range of GSC was found to be rather limited, though A.V. Kiselev et al.[25] and others invested considerable efforts in modifying the adsorbents to enhance the variety of available interactions towards the volatile analytes. Nevertheless, it turned out that even with these modifications the application range of solid stationary phases remained restricted; and mainly suitable for the separation of low-polarity compounds.

Before closing the present section, we would like to refer readers interested in the historic development of GC to a recent review[26], covering most of the early achievements up of the 1950s. Specifically, this article also gives due credit to a range of exceptional scientists from the former USSR for their contributions on this topic.

[5] At the "Institut für Motorenforschung der Luftfahrtforschungsanstalt Hermann Göring".

### *5.4 The Innovation: Gas Liquid Chromatography*

A curiosity in the history of partition GC is the first traceable separation apparently based on gas liquid chromatography and described as early as in 1512, in the period between the Late Middle Ages and the early modern age, by Hieronymus Brunschwig (ca. 1450 - ca. 1512), in his *book "Liber de arte Distillandi de Compositis. Das buch der waren kunst zu distilieren die Composita"*[27] (the title page of this book is shown in Figure 3).

Brunschwig, a German surgeon and botanist, describes a procedure in which the vapor from a mixture of alcohol and water was forced through a sponge moistened with olive oil, and was leading to the recovery of a small quantity of pure alcohol. Expressed in modern terminology, this technique represents a separation process based on frontal GLC, with the oil acting as a liquid stationary phase, the sponge as a porous supporting material, and the alcohol vapor as mobile phase.[28, 29]


**Figure 3.** Title page of the book *Liber de arte Distillandi de Compositis. Das buch der waren kunst zu distilieren die Composita* by Hieronymus Brunschwig, published in 1512, describing a kind of gas liquid chromatography.[27]

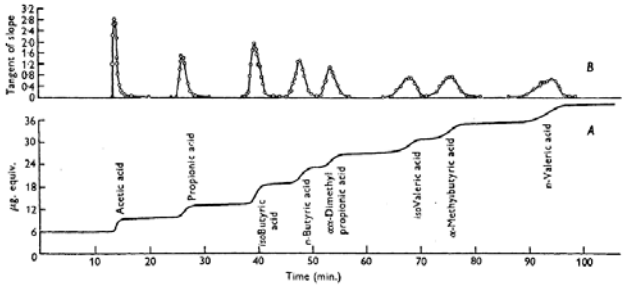


**Figure 4.** Separation of short-chain carboxylic acids by gas liquid chromatography demonstrated in the seminal paper by James and Martin in 1952 introducing partition gas chromatography.[30] Separations were carried out in a packed column in the isothermal mode. Curve A, experimental results; curve B, derivative of experimental curve. For details see Supplementary Information and ref. [30]. From ref. [30] with permission.

However, we will now return to the advancement of gas chromatography as a branch of modern separation science. In the early 1950s James and Martin adopted the concept of liquid liquid chromatography (introduced by Martin and Synge in 1941[16, 17]) to gas chromatography by replacing the solid surface by a liquid as stationary phase, which switched the originally adsorption to partition-based interaction mechanism. In their publications from 1952, James and Martin both suggested a comprehensive theory for gas liquid partition chromatography based on the plate concept (see below), and demonstrated experimentally the separation of volatile acids and bases.[30, 31] Detection and quantitation were carried out by titration of the eluted analytes with an automatic recording burette.

One of their first published separation by GLC[30], that of short-chain carboxylic acids on a column packed with solid-supported poly(phenylmethyldimethylsiloxane) (with 10% w/w stearic acid added) as stationary and nitrogen as mobile phase at 137°C column temperature is shown in Figure 4. Note that all eight acids are completely resolved. Arguably, this invention of partition gas chromatography by using a liquid as stationary phase was the foundation for all further developments in the field, ultimately making GLC to one of the most useful analytical separation methods currently available.

### *5.4.1 Separation selectivity in gas liquid chromatography*

In practical GLC, the vaporized analyte is distributed between the stationary liquid and the mobile gaseous phase with a partition coefficient, $K_i$, which is inversely proportional to the vapor pressure, $p_i^0$, of the analyte as *pure compound* at the given temperature, and to the activity coefficient, $\gamma_i^0$, of the analyte at infinite dilution in the liquid phase. *i.e.* $K_i = prop\ 1/\left(p_i^0\,\gamma_i^0\right)$. We do not give here the derivation of the partition coefficient, but just mention that it can be conveniently obtained by considering Henry's law for non-ideal binary liquid mixtures and Dalton's law of ideal gases (see textbooks about GC).

As in gas liquid chromatography the *selectivity coefficient,* $r_{ji}$ is expressed by

$$r_{ji} = \frac{k_j}{k_i} = \frac{K_j}{K_i} = \frac{p_i^0}{p_j^0}\frac{\gamma_i^0}{\gamma_j^0} \tag{7}$$

and as $r_{ji}$ must be larger than unity, it follows from Equation 7 that a given pair of analytes can be readily separated if their products of $p^0$ and $\gamma^0$ differ. For chemically very similar analytes - with about equal $\gamma^0$ - this can be achieved if the vapor pressures, $p_i^0$ and $p_j^0$ of the pure compounds differ at the operational column temperature. However, more relevant for the variation of separation selectivity is the ratio of the activity coefficients $\left(\gamma_i^0/\gamma_j^0\right)$ because it reflects the specific intermolecular interactions between the analytes and the liquid stationary phase.[32, 33] Thus, by proper selection of stationary phases liquids from a broad range of chemically distinct compounds, the activity coefficients of given pair of analytes can be conveniently adjusted so as to achieve the level of selectivity required for a given separation.

### *5.4.2 Isothermal and temperature-programmed mode*

In GLC, the *isothermal elution* mode (in which the column is held at constant temperature) is suitable for the separation of sample constituents which possess retention factors within a reasonably narrow range. At a given constant column temperature, the vapor pressures, and the activity coefficients of the sample components, and therefore their retention factors, all remain essentially constant during the chromatographic run. Consequently, the selection of an appropriate operational column temperature enables the adjustment of the elution of the analytes of interest to an acceptably narrow retention time window.

However, isothermal conditions are not favorable for the separation of samples composed of analytes with largely differing retention factors. For such mixtures, at a low column temperature the early eluting analytes can be satisfactorily resolved, while those possessing very large retention factors will elute at unacceptably long retention times. Moreover, the longer the retention time

the broader the peaks become (see Chapter 4.2), and the late-eluting wide peaks might even disappear within the noise of the baseline of the chromatogram. Selection of a high temperature, on the other hand, would be an efficient means to adjust the retention characteristics of the late eluting compounds appropriately, yet with the drawback that under these conditions the early eluting compounds would be poorly retained and can thus emerge from the column unresolved (see the middle term of the resolution equation, Equation 6). Certainly, isothermal GC is not a beneficial method for the analysis of mixtures with such complex compositions.

This general elution problem is valid for all chromatographic techniques, *i.e.* GC and LC. For GLC this fundamental issue can be conveniently addressed by exploiting the strong temperature dependence of the distribution coefficient and the retention factor, respectively. Pioneering investigations of the chromatographic behavior of compounds in adsorption columns in presence of longitudinal temperature gradients were carried out by Turkeltaub, Zhukhovitskii, et al.[34, 35]. The authors coined for this separation method the term chromathermography.

In GLC, the vapor pressure of the pure compound increases exponentially with increasing temperature (according to the Clausius-Clapeyron equation), and accordingly $K_i$ and $k_i$ decrease exponentially with increasing temperature. Exploiting these facts, in 1958 S. Dal Nogare et al.[36, 37] introduced a method to run samples consisting of components with large differences in volatility by varying the column temperature, $T$, as function of time, $t$, *i.e.* by applying a certain gradient $dT/dt$ to the entire column. When employing this *temperature programming* technique, the initial low-temperature conditions are adapted to ensure appropriate retention of the early eluting analytes, while the final high temperature conditions are chosen to enable complete elution of the late eluting sample components. Between these limits, the retention factors of the analytes are continuously decreases by the action of the well-defined $T$-gradient, with the consequence that the observed retention times are significant shorter than those seen under isothermal conditions. Apart from reducing the time of analysis, temperature programming also causes a pronounced compressing of the analyte zones into very sharp peaks. [38, 39]

### *5.5 GLC with Packed Bed Columns: Low Efficiency and Needs for Selectivity*

Early analytical GC columns were fabricated from metal or glass tubes with inner diameters of several millimeters and few meters in length. They were packed with porous solid particles (initially e.g. granules of firebrick, later kieselgur, *i.e.* purified diatomaceous earth), which were impregnated with the stationary liquid prior to use. The early versions of supporting material were later replaced by well-defined commercially produced homogeneous synthetic particles. Packed bed columns (also referred to as *packed columns*) could easily be prepared, which favored their general acceptance.

As already mentioned, the separability of analytes is impaired by a number of processes caused by the inevitably broadening of the initially narrow sample zone, processes that determine the column efficiency. In the first theoretical approach to describe band broadening, formulated by the *plate theory*, the column is considered as being composed of a series of interconnected cells or "plates" containing the mobile and the stationary phase. Upon migrating through the chromatographic column, the analyte is assumed to distribute between these two phases within each plate element with equilibrium being reached.[40] The mobile phase with the fraction of solute at equilibrium concentration is then transferred to the next plate downstream, where the same process takes place again. It is important to recognize that the plate theory assumes that at each distribution step equilibrium conditions are achieved. This requirement is certainly not fulfilled as during the chromatographic process in the column the fraction of the solute in the mobile phase is continuously transported by the mobile phase while the fraction in the stationary phase permanently lags behind. Equilibrium would only be achieved under the condition of infinitely fast inter-phase mass transfer, which is an unrealistic proposition. This means that analyte distribution actually occurs under non-equilibrium conditions[41, 42], and therefore the kinetics of the mass exchange will additionally contribute to the "*Height Equivalent of a Theoretical Plate*" (HETP; the terminology is adopted from the plate theory). Logically, this particular contribution will become the more pronounced the higher the migration velocity of the zone is. In contrast, at sufficiently low migration velocity the inter-phase mass exchange will approach equilibrium conditions. This additional contribution to zone dispersion is taken into account in the *rate theory*, which specifically accounts for the effects of finite inter-phase mass transfer kinetics.

The rate theory for packed columns, considering the plate height, $H$, as function of the velocity, $v$, of the mobile phase, was formulated by van Deemter, Zuiderweg and Klinkenberg [43] (for elution chromatography and for isothermal conditions), expanding the theories described by Lapidus and Admunson[44], and by E.

Glueckauf[42]. In its simplified form, $H=f(v)$, is expressed by the three-term function

$$H=A+B/v+C.v \quad (8)$$

For GLC with packed columns term $A$ reflects the contribution to peak broadening caused by eddy dispersion due to the heterogeneous particle-size distribution of the packing, term $B$ describes the contribution of longitudinal diffusion and of the different path lengths of the flow lines around the particles, term $C$ accounts for the kinetics of the mass transfer of the solute molecules between the phases. Note that this equation contains objective quantities like retention factor, particle diameter, diffusion coefficients, but also several empirical factors, *i.e.* those characterizing packing geometry and tortuosity. For more complex conditions the rate theory was refined by J.C. Giddings.[39, 45]

According to the van Deemter equation (Equation 8) $H$ decreases hyperbolically with increasing $v$ considering term $B/v$, while it increases linearly with $v$ with regard to term $C.v$ (term $A$ is independent of the velocity). The resulting $H$ *vs.* $v$ curve, given by the sum of the individual curves, exhibits a minimum plate height, $H_{i,min}$, at a distinct, singular flow velocity; *i.e.* at this flow velocity the column will produce maximum efficiency for a given analyte (note that $H_{i,min}$ depends on the retention factor, and therefore the different analytes have different values of $H_{i,min}$ even under identical conditions).

In their seminal contribution, van Deemter et al. put their theory on test by measuring $H=f(v)$ curves for a number of analytes and columns, and the results obtained for *n*-butane and *i*-butane are shown in Figure 5.[43] The $H$ *vs.* $v$ curves show shapes that are in accordance with theory, *i.e.* curves exhibiting a minimum at a singular carrier gas velocity, and distinct $H_{i,min}$ for the individual analytes.

As a consequence of their inherently low efficiency packed columns produce relative broad peaks, which can easily be rationalized using the general expression for the plate number $N=L/H$. Obviously, one reason for the low plate number is the considerably large value of $H$ generally observed for packed columns. The second reason is the relatively short column length (of about a few meters), which is a practical limitation dictated by the need to keep the inlet pressure of the carrier gas workably low. Plate numbers are therefore rarely higher than a few thousands. Given this limitation in efficiency, considerable research was devoted to improving separation performance of packed column GLC through optimization of selectivity.[32, 33] This goal was met by employing a large number of the stationary liquids with a wide range of polarity, an effort that led to the development of several hundred commercially available stationary phases with optimized yet rather narrow selectivity profiles.

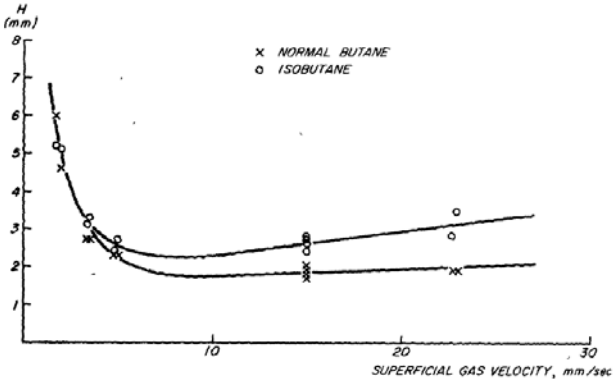


**Figure 5.** Plate height, $H$, in dependence on the mobile phase velocity, taken from the original publication of van Deemter, Zuiderweg and Klinkenberg from 1956. Measurements were carried out with a packed bed column under isothermal conditions. For details see Supplementary Information and ref. [43]. From ref. [43] with permission.

### *5.5.1 Analyte identification by GC: Retention or Kováts Index*

In the early days of GLC, rather unspecific detection systems (instruments coupled with mass-sensitive detection devices were yet to come) were employed, rendering the identification of an unknown analyte, *y*, challenging. To address these issues, concepts were developed that allowed identification of unknowns based on their chromatographic behavior by comparing the retention characteristics with those of known reference compounds listed in the literature. The most obvious choice of parameter for this purpose, their retention time, is not sufficiently robust, as it depends on a number of instrumental variables, such as the mobile phase flow velocity, the length of the column, the phase ratio, and the temperature. The retention factor, $k_y$ of the unknown analyte, *y*, being independent of flow velocity and column length, is a better choice, yet is still a function of the phase ratio. However, the variation of the retention factors with different phase ratios can be accounted for by resorting to relative retention factors, *i.e.* retention factors that are calibrated using a set of reference compounds.

For GLC, E. Kováts[46] proposed the homologue series of straight-chained alkanes as suitable reference compounds, and defined the so-called Retention (or

Kováts) Indices, $I_{R,n}$, for all stationary phases (and at all temperatures) as exactly the hundredfold of the number of their C-atoms: $I_{R,n}$=100.$n$; normal undecane, e.g. has an Index of 1100. An analyte, $y$, which elutes between two homologue straight-chained alkanes with carbon numbers of $n$ and ($n$+1) is considered to behave like a hypothetical alkane with C-number $n_y$, a fractional number between $n$ and ($n$+1). This hypothetical number $n_y$ can be calculated from $k_y$, given the linear dependence of log$k_n$ on $n$ of the reference alkanes (this relationship is strictly valid under isothermal conditions only); the analyte-specific Kováts or Retention Index results therefore $I_{R,y}$=100.$n_y$.

Retention indices depend on the stationary liquid only and thus enable the identification of unknown analytes by comparing experimentally measured values with those documented in the literature (huge collection of $I_R$ values for many compounds and stationary phases have been compiled over the years and were readily available in the literature and databases). Identification of unknown compounds is considerably facilitated by comparison of indices measured on several different stationary phases. If no reference indices are available, the difference of retention indices, $\Delta I_R^{p/ap} = I_R^p - I_R^{ap}$, measured on a polar ($p$) and an apolar ($ap$) phase can be exploited to gain information about the type of the functional groups present in the analyte.

It should be mentioned that this concept was successfully applied to the analysis of binding media of the paste layer of a shell-inlaid ceremonial shield from the Solomon Islands.[47] The object originates from the 1st half of the 19th century, and is housed in the Weltmuseum (the former Museum of Ethnology) in Vienna, Austria. In the course of the investigation of the composition of paste layer two sample constituents were detected by GC, but compound identification by MS was hampered by observation of essentially identical mass spectra (at least with MS instrumentation available at the time the study was carried out). However, these two analytes could be identified as two isomeric octadecatrienoic acids by means of their $\Delta I_R^{p/ap}$.

### *5.5.2 Polarity of stationary phases: Rohrschneider-McReynolds Index*

*Polarity* is a term employed to describe the chromatographic retention characteristics of a stationary phase. Initially used rather intuitively, L. Rohrschneider[48] and later W.O. McReynolds[49] introduced a concept to codify the polarity by a number. This concept is based on the Retention Index differences, $\Delta I_R^{p/Sq} = I_R^p - I_R^{Sq}$, of certain selected reference compounds on a given stationary phase, $p$ , relative to that of a highly apolar stationary phase, with the latter being squalane ($Sq$), a branched C30 alkane. Initially five reference compounds (benzene, ethanol, ethyl methyl ketone, nitromethane, and pyridine) were selected by Rohrschneider (the set was later extended to ten reference compounds by McReynolds) to represent characteristic types of interaction with the liquid phase (*i.e.*, London dispersion, π– π electron, electron attracting and dipole-dipole interactions, H bonding capability). The $\Delta I_R^{p/Sq}$ values represent measures for individual intermolecular forces of these reference compounds with the stationary liquid, and are expressed for practical applications as constants $x', y', z', u', s'$. Under the assumption that the retention behavior of a stationary phase is a manifestation of its intermolecular interaction forces, the sum of the constants, $\Sigma = x' + y' + z' + u' + s$ generally known as the Rohrschneider-McReynolds Index, Σ is a specific measure for the polarity of a given stationary liquid.

This index can be used to rank the stationary phases according to their polarity; e.g., Σ is zero for squalane, 229 for relatively apolar poly(dimethylsiloxane), and 3682 for highly polar poly(cyanopropylphenylsiloxane). In practice, these Indices are particularly helpful for the assessment of the similarity of the polarity for stationary phases from different commercial sources. Moreover, the Rohrschneider-McReynolds Index and constants can be used to guide the selection of appropriate stationary phases for the separation of given analytes [6].

## 6. THE INCLUSION OF GAS LIQUID CHROMATOGRAPHY WITH PACKED COLUMNS TO THE ANALYSIS OF BINDING MEDIA

From about 1965 the potential of GC for the analysis of binding media of museum objects was started to be recognized, although this technique was not directly applicable to a number of substance classes of present interest. Plant gums (polysaccharides) or animal glues (protein) would rather decompose than evaporate at high temperature, but appropriate procedures for their transformation into GC-conform modifications were developed or adapted from the literature. However, some problems specific to the analysis of museum object still

[6] Such selections are often guided by the well-known rule-of-thumb "*similia similibus solvuntur*" concept, which may be understood as the three-word essence of the Rohrschneider's polarity classification. It appears to have been formulated in analogy to the principle "*similia similibus curantur*", attributed to Paracelsus, and "*similia similibus curentur*", a motto of homoeopathy (for the source of the solubility rule see J.H. Hildebrand, R.L. Scott, *The Solubility of Nonelectrolytes*, ACS Monograph No. 17, Reinhold Publ. Corp., 1950).

have to be overcome, such as the very limited sample amounts available, the complexity of mixtures of several classes of binders often encountered in a single sample, occasionally together with products stemming from degradation and decomposition processes, the large excess of organic and/or inorganic matrix compounds and the presence of contaminants. Much research was devoted to address these issues over the second half of the 1960s.[50-53] Especially J.S. Mills and R. White carried out a number of systematic investigations concerning GC analysis of the different binding media[51, 54-59], and published later a comprehensive account on this topic.[2]

First GC analyses of binding media were conducted with packed columns. An example for a chromatogram obtained under isothermal conditions for resinous material present in wax models is shown in Figure 6, top panel. It is worth mentioning in this context that the results of modern analytical efforts can be supported as described in detail in Johann Melchior Cröker's book "*Der wohl anführende Mahler …*" which appeared in 1743. In the part attached to this volume entitled "*Diesem ist noch beygefüget ein Kunst-Cabinet rarer und geheim gehaltener Erfindungen,...*" Cröker disclosed detailed recipes *("…rare and secret inventions,…")* concerning the fabrication of colored wax and recommended addition of Venetian turpentine or, in some cases, Cyprian turpentine.

The chromatograms shown in Figure 6B, top panel, were obtained from an anatomic wax model dating from the 18th century, and in Figure 6C, top panel, from a wax model known as "Christ rejected by the Jews", created ca. 1579 by Giovanni Bologna. Peaks were attributed to resin acids (actually their methyl ester; the binding media were subjected to methylation prior to analysis to improve volatility) based on comparison with reference diterpenoic resins. Note that the detection of larixyl acetate points to the presence of Venetian turpentine as additive to the wax. The same resin was detected by capillary GC in samples taken from an anatomic wax model belonging to the collection of the History of Medicine in Vienna, Austria, created in 1786 in the workshops of the famous anatomists F. Fontana (in Pisa) and P. Mascagni (in Florence).[60]

Certainly, the peaks of the chromatograms depicted in Figure 6B and 6C (both top panel) are relatively broad, a feature which is characteristic for packed column GC, and their widths increase significantly with increasing retention time, inherent to isothermal conditions. As pointed out previously, elution at constant column temperature leads to unsatisfactory long times of GC analysis for samples consisting of components differing widely concerning volatility. Natural waxes are good

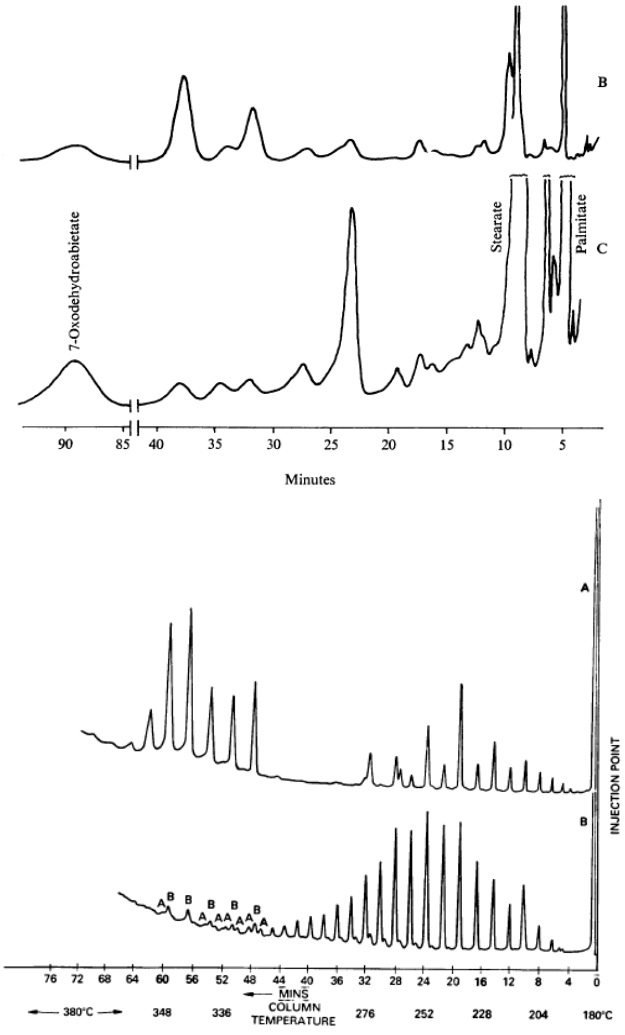


**Figure 6.** Comparison of packed column GC in isothermal with *T*-programmed mode. *Top panel:* Isothermal GC of resinous material in (B) an 18th century anatomic wax model; (C) the wax model "Christ rejected by the Jews" by Giovanni Bologna, ca. 1579[58] (chromatographic conditions as in ref. [54]). Peak at 23.5 min, dehydroabietate; 37.5 min, larixyl acetate. For details see Supplementary Information and ref. [58]. From ref. [58] with permission. *Bottom panel:* T-programmed GC. Samples: Waxes from (A) a surface coating of a 15th century Intarsia work; contains bees wax, small proportion of ceresin wax; (B) 18th century Italian wax sketch; contains a mixture of ozokerite with traces of bees wax (esters marked B). For details see Supplementary Information and ref. [59]. From ref. [59] with permission.

examples for such mixtures; e.g. beeswax, which mainly consists of straight-chained hydrocarbons with 21 to 33 carbon atoms, and long chain esters with triacontanyl palmitate (containing 46 carbon atoms, see also Figure 1) being the most abundant one. It is obvious that for samples with such a composition application of tempera-

ture programmed GC provides the combined advantages of reduced analysis time and narrow peak shapes. The benefits of temperature programmed GC are evident in chromatograms given in Figure 6A and 6B (both bottom panel), both of which were obtained on a packed column[59]. The chromatogram in Figure 6A (bottom panel), was obtained from a sample taken from a surface coating of an Intarsia work dating from the 15th century, with beeswax being the main constituent. The chromatogram in Figure 6B (bottom panel) represents the compound profile of a sample taken from an Italian wax sketch dating from the 18th century, providing evidence for the presence of ozokerite, a naturally occurring wax consisting mainly of long-chain *n*-alkanes.

## 7. THE BREAKTHROUGH TO HIGH EFFICIENCY: CAPILLARY COLUMNS

The transition of packed bed to capillary GC, an event that marks undoubtedly a major milestone in the evolution of GC, is closely connected with the name of Marcel J.E. Golay. Golay was a Swiss electrical engineer and mathematician; after having joined Bell Laboratories, he worked at the U.S. Signal Corps Engineering Laboratories for 25 years before affiliating with a leading instrument company aiming to develop a multiple slit IR spectrometer. Remarkably, while Golay had no previous involvement in chromatography[61], stimulated by the discussion of colleagues engaged in GC, he took interest in the mathematics describing the dispersion processes in the packed columns. Through a critical analysis of the basic assumption of the underlying theory, Golay concluded that the irregular pathways the analytes have to negotiate upon their passage through the particle beds are the main source of low efficiency of packed columns. Golay suggested that this deleterious effect may be avoidable by employing straight parallel open tubes (with a thin film of the stationary liquid phase coating their inner wall) rather than a packed bed giving rise to meandering channels. Within few months, he succeeded in working out a theory of chromatographic dispersion in open tubular columns, and suggested to test his theoretical predictions by experiment. In addition, to facilitate this efforts, he constructed a low-volume thermal conductivity detector better suited for the capillary column [7] he used (12 ft in length, with 0.055 inner diameter (i.d.), *i.e.* 366 cm x 1.37 mm) than the contemporary large-volume devices. One of the first chromatograms

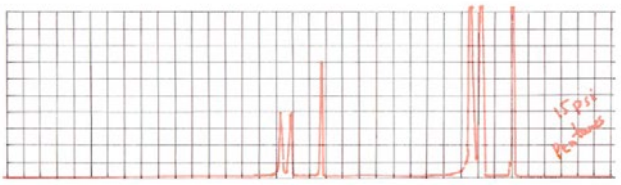

**Figure 7.** M. Golay´s one of the first chromatograms with a capillary column measured in 1956, separating a mixture of isomeric pentanes. Column: 366 cm length, 1.37 mm i.d.; stationary phase (coating the inner capillary wall), polyethyleneglycol; isothermal at room temperature. Detection with miniaturized thermal conductivity detector. For details see Supplementary Information and ref. [61]. From ref. [61] with permission.

Golay obtained with capillary columns is shown in Figure 7, providing compelling proof that his predictions concerning the improvements in separation efficiencies were consistent with the physical realities.

Golay disclosed his theory and the supporting experimental results in 1958[62-64], and thus sparked a revolution in terms of further developments in gas chromatography. His results were successfully reproduced and confirmed by other leaders in the field, e.g., by D.H. Desty et al.[65, 66] and by R.P.W. Scott[67], and capillary columns went on to quickly replace packed bed columns essentially for all but preparative applications.

Given the impact Golay´s contributions made on the state-of the-art in GC, it appears justified to review some important aspects of his mathematical description of the dispersion phenomena in capillary columns. For an open tube of cylindrical geometry with a film of liquid deposited at its inner surface and with a gas flow velocity, $v$, Golay established the following equation for the dependence of $H_i=f(v)$

$$H_i = \frac{2D_{m,i}}{v} + \frac{\left(1+6k_i+11k_i^2\right)}{\left(1+k_i\right)^2}\frac{r^2}{24D_{m,i}}v + \frac{2}{3}\frac{k_i}{\left(1+k_i\right)^2}\frac{d_s{}^2}{D_{s,i}}v \quad (9)$$

This equation can be expressed in a simplified form as

$$H_i = B/v + \left(C_m + C_s\right)v = B/v + C\,v \quad (10)$$

The first term, $B/v=2D_{m,i}/v$ in Equation 9 stands for the contribution to peak broadening caused by longitudinal diffusion of the analytes in the mobile phase ($D_{m,i}$ is the diffusion coefficient of analyte, $i$, in the mobile phase). The middle term in Equation 9 ($C_m$ in Equation 10) expresses the combined contributions of the parabolic flow profile in the cylindrical tube (with inner radius,

[7] Nowadays these columns are generally named *capillary columns*, although Golay preferred the term *open tubular columns*, because narrow tubes - capillaries - could in principle also be packed with particles.

*r*) and the diffusion in radial direction which is part of the mass exchange in the mobile phase. Its contribution is weighted by the factor which is solely $k_i$-dependent. The contribution of the kinetics of mass exchange from the stationary phase is expressed by the third term in Equation 9 ($C_s$ in Equation 10); it depends on the film thickness $d_s$ on the diffusion coefficient in the liquid phase, $D_{s,i}$, and on $k_i$. For columns with very thin films this term plays a less pronounced role.

The simplified form of the Golay equation (see Equation 10) resembles that of the van Deemter equation for packed columns, with the exception that it lacks term *A* (simply because the column contains no packing) and the *C*-term does not feature any empirical parameters. Compared with packed columns, Golay`s approach predicts higher plate numbers for open tubular columns for two reasons: i) the attainable plate heights *H* are lower, and ii) the absence of a packing results in lower flow resistance and therefore much longer open tubes can be employed than is possible with densely packed columns. Consequently, the plate number $N=L/H$ is larger. Since open tubular columns can be operated with lengths of up to 100 m, their plate numbers can reach several hundred thousands, being larger by nearly two orders of magnitude as compared to the plate number achievable with packed columns.

From the discussions given above it can be concluded that the mobile phase velocity is an important parameter for adjusting the efficiency of a column in practice. It shall be mentioned that, since gases are viscous and compressible media, the flow velocity is not constant but is a function of the radial and of the axial position in the column. However, the fact of the different axial flow velocity will not be further discussed here, as under the usual operation conditions it is a factor of minor significance. If being of interest, pressure-dependent velocities can be calculated and accounted for by appropriate correction factors as described by James and Martin[31] or retrieved from the literature.

### *7.1 The Improvement of the Capillary Material*

#### *7.1.1 Metal and glass capillaries*

The early capillary columns were fabricated from metal, such as copper, nickel, alumina or, preferentially, from steel, or from synthetic organic polymers, and different methods to implement the stationary phase were developed. However, metal columns suffered from some disadvantages, e.g. considerable activity and the unevenness of the inner surface, giving rise to band distortion and loss of separation efficiency. Therefore, metal capillary columns were rapidly abandoned in favor of glass capillaries after D.H. Desty et al.[68] had succeeded in the development of a device to draw reliable capillaries from a molding blank.

In the currently most common column type the capillary wall is directly coated with a thin film of liquid. However, initially, with glass capillaries the direct coating approach was met with difficulty due to the low wettability of the solid surface (especially for apolar liquids) and its undesirable adsorptive properties. Adsorption at the glass surface, caused by the presence of higher-charged cations, led to a severe distortion of the peaks with a distinct tailing, resulting from nonlinear adsorption isotherms of the solutes interacting with the liquid-solid interface. Adsorption-triggered tailing occurred especially in the case of polar analytes containing donor/acceptor functionalities. A typical example of adsorption-induced peak distortion on a non-deactivated glass surface reported by Schomburg et al.[69] is given in Figure 8. Note that in this particular case, symmetrical peak shapes were obtained after deactivation of the glass surface.

To improve the quality of glass surfaces, special treatments were necessary prior to conducting the coating procedure. Thus, wettability was enhanced by increasing the roughness of the surface by acidic etching, e.g. using dry gaseous HCl or HF.[70] For the deactivation of the surface a number of other procedures were advanced, e.g. the deposition of polymer layers, or the exhaustive silylation of surface silanol groups.

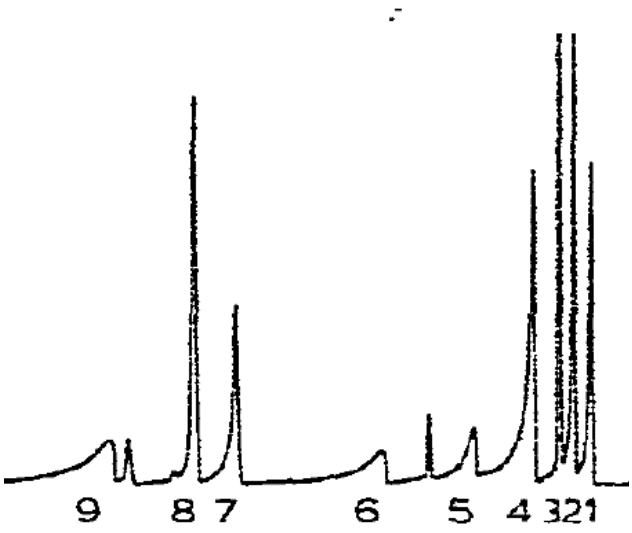


**Figure 8.** GC with glass capillary without surface deactivation prior to coating with polyethyleneglycol. Analytes 1-9: alkylamines. Figure taken from ref. [69] with permission and modified.

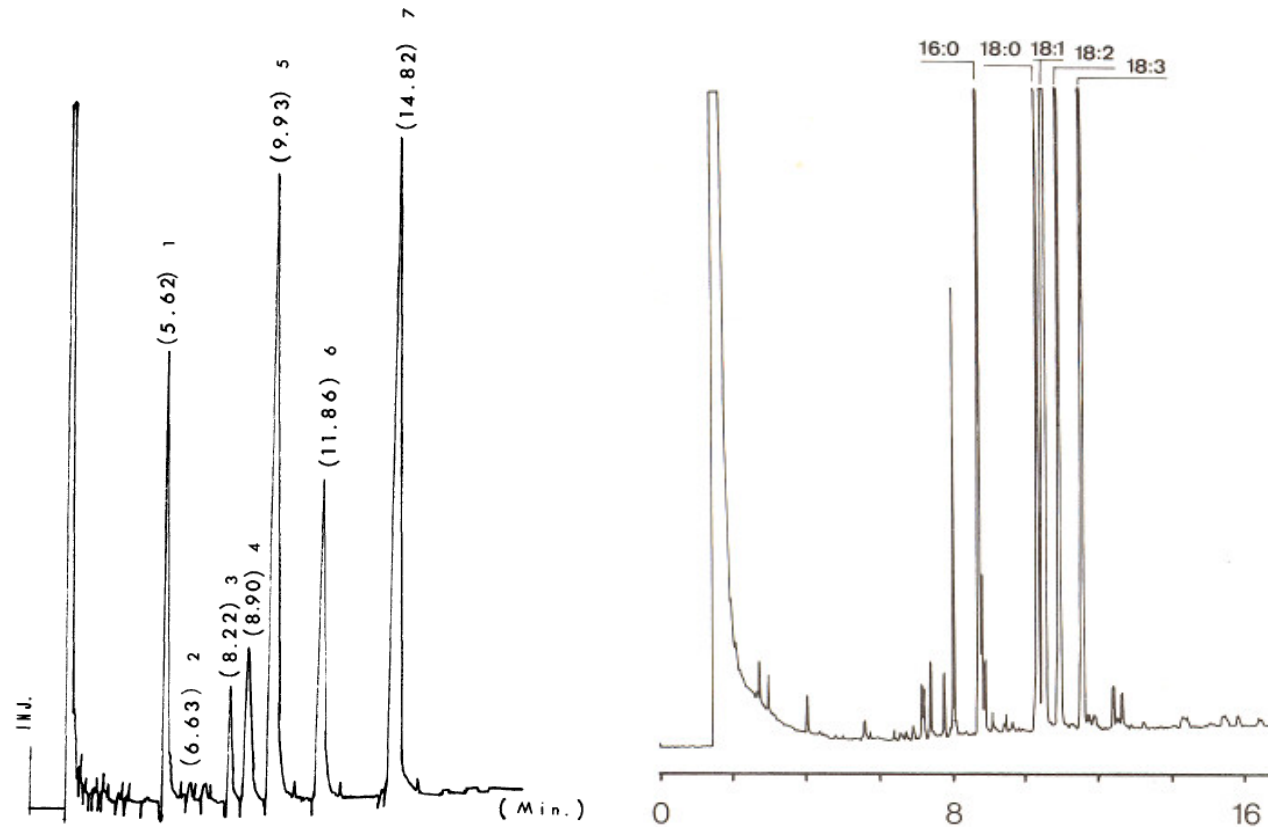


**Figure 9.** *Left panel:* Early GC with a glass capillary (isothermal mode) for binding media analysis (drying oil). Samples: fatty acids (as methyl esters). Numbering of acids: 1, palmitic (16:0); 2, suberic; 3, azelaic; 4, stearic (18:0); 5, oleic (18:1); 6, linoleic (18:2); 7, linolenic (18:3). For details see Supplementary Information and ref. [71]. From ref. [71] with permission. *Right panel: T*-programmed capillary GC. Sample: fatty acids (as methyl esters, after hydrolysis of the sample). Sample taken from the paste layer of a ceremonial shield from the Solomon Islands (collected mid-19$^{th}$ century). Time in min. For numbering of analytes see Legend of left panel. For details see Supplementary Information and ref. [47]. From ref. [47] with permission.

In Figure 9, left panel, one of the first applications of glass capillary columns in the area of binding media analysis is shown, namely for the separation of fatty acids (converted into their methyl esters prior to analysis) from a sample of drying oils. The glass capillary was coated with a polar stationary phase and GC was carried out in the isothermal mode. All relevant analytes are separated. Yet, the peak widths are generally broad, and the analytes migrate over a relatively wide retention time window of about 10 min.

In contrast, in Figure 9, right panel, a temperature-programmed analysis of the same group of analytes is shown, originating from the paste layer of a ceremonial shield collected mid-19$^{th}$ century at the Solomon Islands (mentioned in Chapter 5.5.1)[47], demonstrating the inherent advantages of this elution mode. Specifically, here the fatty acid profile (also as methyl esters) elutes within a retention time window of about 4 min, with all compounds emerging as narrow peaks (Note that in this case the relatively long overall run time was chosen deliberately to accommodate for potentially other unknown components in the sample).

Even though glass capillary columns found widely use in GC due to their high efficiency, some problematic properties remained. One of these - but not the most relevant - was their poor mechanical stability, which complicated their handling and installation, especially in context with the coupling to mass spectrometers. However, much more problematic was the fact that the coated glass surfaces, despite of careful surface pretreatment, often retained a certain level of active adsorptive sites. In efforts to address these issues, rather sophisticated protocols were developed for deactivation, coating and stabilization of the stationary phases, involving the cross-linking between polymer chains of the liquid stationary phase to obtain immobilized phases, and the

covalent anchoring of stationary phases onto the capillary surface to create bonded phases. Major contributions in the field of the stationary phase chemistry in context with glass (and in the next generation of fused silica) capillary columns[61] are connected with the names of L. Blomberg[72], K. Grob[73], M.L. Lee[74], S.R. Lipsky[75], C. Madani[76], V. Pretorius[77], P. Sandra[78] and G. Schomburg[79].

### *7.1.2 Fused silica capillaries*

In the course of their pursuit of more suitable glass materials for capillary GC applications, R. Dandeneau and R.H. Zerenner discovered that fused silica proved the most inert material.[80] After their initial demonstration of the superiority of fused silica over glass capillaries for GC applications, this material was intensely investigated and further popularized by S.R. Lipsky et al.[75] and others. At his time, the technology for the fabrication of synthetic fused silica was already well established in the field of fiber optics, and could be readily adapted for the production of capillaries with little additional effort. Industrially, fused silica is produced by hydrolysis of $SiCl_4$ in the gas phase and subsequent melting of the resulting high purity $SiO_2$. The emerging material contains about 0.1 ppm metal oxides as impurities only, as opposed to naturally occurring quartz, for which the content of metal ions is typically higher by two orders of magnitude. Due to this high purity, stationary phases coated or immobilized in fused silica capillaries were much more stable thermally, and showed much reduced adsorptivity for polar analytes as compared to other capillary materials. Furthermore, residual activity due to silanol groups could effectively be suppressed using silylation procedures established in the past for glass surfaces.

Most procedures originally developed for the fabrication of immobilized and bonded phases in glass capillaries (see above) could be directly adopted to the fused silica material. Bonded phases, in many cases polysiloxanes derivatives, possess a number of advantages. Due to their attachment at the surface, they are resistant to extraction with organic solvents, and thus do not become detached upon direct injection of samples dissolved in polar solvents and even in water. Furthermore, these columns show, yet at high operating temperatures, negligible bleeding (*i.e.* less release of lower molecular mass constituents of the stationary phase at elevated temperature). Also, they exhibit chemically excellent long term stability, and maintain over extended periods of use their chromatographic performance in terms of retention and efficiency. Given these advantages fused

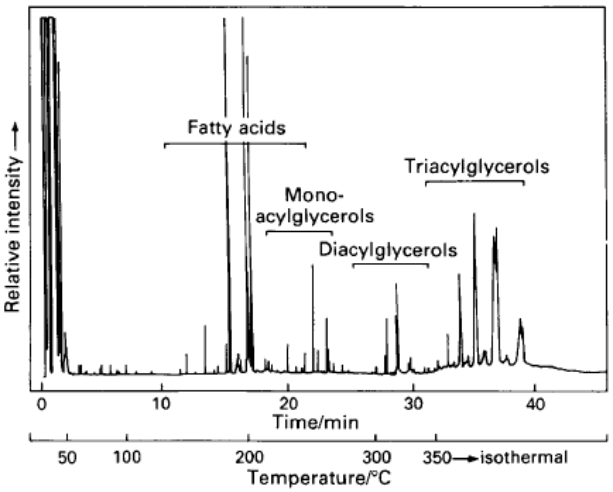


**Figure 10.** High-temperature GC. Analysis of intact fatty acid mono-, di- and triglycerides. Samples extracted from a sherd recovered from an Early Medieval ditch. Alumina cladded fused silica capillary column, immobilized stationary phase, end temperature 350°C. For details see text, Supplementary Information and ref. [81]. From ref. [81] with permission.

silica capillary columns completely replaced glass-based columns for most but some specialty application (e.g. for chiral separations) for which glass capillaries are still the preferred material.

Especially designed stationary phases featuring remarkable thermostability allow analysis of high molecular compounds. A typical application of high temperature GC is shown in Figure 10, for profiling lipids contained in a sherd recovered from an Early Medieval ditch.[81] In this particular case, GC analysis was conducted in a temperature-programmed fashion with an upper temperature of 350°C, using a fused silica capillary containing a bonded apolar stationary phase. Analytes were intact mono-, di- and triglycerides, which were directly subjected to analysis without any prior hydrolysis/derivatization (Note that other sample constituents featuring free carboxylic and hydroxyl groups were silylated prior to analysis).

## *7.2 The Development of Sample Injectors required for Capillary Columns*

The plate heights expressed by the van Deemter equation for packed columns and the Golay equation for capillary columns account only for zone broadening processes occurring within the chromatographic columns. In other words, it is assumed that the length of the injected sample plug and therefore the standard deviation of the input function approach zero. In prac-

tice, however, the injected zone has a finite length and consequently contributes to the final peak width. If, e.g., the injected plug is rectangular sized with length $\delta$ its variance is $\sigma_{inj}^2 = \delta^2/12$ in the length domain). The standard deviation of the final peak is then, according to the general additivity of variances, equal to $\sqrt{\sigma_{inj}^2 + \sigma_{col}^2}$ with $\sigma_{col}^2$ being the variance caused by the dispersion processes within the chromatographic column.[82] Zone dispersion contributions from sources outside of the separation column, such as the injected plug and the detector volume, are generally referred to as *extra-column effects*, and inevitably impact the experimentally observable peak width. However, the impact of these generally unfavorably extra-column contributions on the overall performance is quite different in severity for packed and capillary columns, respectively.

When using packed columns, samples are generally introduced by an injector which consists of an evaporation chamber, a heated cylindrical glass or quartz tube with about one mL volume. The injector is coupled with an external gas supply which provides a continuous flow of the mobile phase carrier gas. At the outlet of the injector the packed column is mounted, and the inlet of the injector is typically sealed by a silicon rubber septum. The solid or liquid sample is dissolved in a highly volatile solvent, and is injected through the septum by a syringe into the evaporation chamber and where it is flash-vaporized. Here the vapor is homogeneously mixed with the gas phase before it flows into the packed column. Typically, the injected volumes of the liquid sample solutions are in the microliter range, being volumes that can conveniently be handled by precision syringes. These volumes cause a considerable large injected zone of vapor at the column inlet, and typically contain a relatively large amount of sample. As pointed out earlier, this zone contributes to the width of the final peak in addition to the dispersion produced within the column, However, due to the inherently low efficiency of packed column, the extra-column contributions associated with sample introduction remain rather negligible relative to the significant in-column dispersion. In addition, mass overloading of the packed column by the relatively large amount of analyte does usually not occur due to the large volume of the stationary phase available in the column (more currently due to the large phase ratio).

While the described type of injection device is well suitable for sample introduction into packed columns, it is incompatible with the requirements of capillary columns. Firstly, a large input sample zone would dominate in-column peak broadening and thus obscure the high efficiency of the capillary column. Secondly, introduction of a large amount of analytes would certainly mass-overload the column as result of its small phase ratio (the inner diameter of the capillary is about 200-300 micrometer, the thickness of the stationary liquid is only in the micrometer range or lower) and thus give rise to severely leading peak profiles. Therefore, the only way to preserve the inherently high efficiency of capillary column consists in introducing suitably small sample amounts in terms of mass and volume. However, as liquid volumes smaller than about 0.1 microliters are hard to handle by syringes, capillary column generated the need for especially designed sample introduction devices, e.g. allowing for a partial injection of the sample vapor after being mixed with the carrier gas.

This was achieved by engineering injectors that split the gas flow between an additional outlet and the column. Typically, the outlet consisted of a restrictor which enabled adjusting the desired split ratio between column and the vent. In practice, split ratios (vent to column) were selected between several 10:1 and few 100:1. In this way, extra-column peak broadening and sample overload of the capillary column can be avoided.

This simple split-injector design, developed in the 1960s, and still in use nowadays, has some inherent disadvantages, e.g. the marked loss of analyte (via the vent) and the consequent loss of sensitivity of the analytical method. To address these limitations, other, more sophisticated injector types were developed, e.g. the splitless (see e.g. ref. [83]), the Programmed Temperature Vaporizing (PTV)[84] and the on-column injectors. However, these developments will not be described here.

In this context it must be pointed out that with the advent of the capillary column also new sensitivity requirements for the detecting systems arose. For packed columns initially the thermal conductivity detector was commonly in use and yet effective. Its relatively large volume translates technically into low sensitivity, but given the large injected sample typically processed in packed column GC provided satisfactory signal strength. However, the typical sample amounts eluting from capillary GC are much smaller, and would produce a low if any signal with the traditional detection systems. It was therefore a fortunate coincidence, that Golay´s introduction of capillary GC was synchronized with the first detailed technical description of the highly sensitive flame ionization detector (FID)[85], which could easily be miniaturized to meet the new demands. This detector responds to CH-groups and is, by the way, thus perfectly suitable for organic compounds of the binding media.

## 8. PYROLYSIS GAS CHROMATOGRAPHY

As mentioned previously, certain classes of non-volatile compounds can be addressed by GC analysis after being degraded into smaller volatile fragments through chemically well-controlled reactions (e.g. for proteins and polysaccharides through acid hydrolysis). However, this conventional approach is inapplicable to materials for which no suitable degradation reactions exist (e.g. high molecular mass condensed hydrocarbons, the main constituents of bituminous materials). For these non-volatiles, pyrolysis GC offers an attractive option for direct analysis. This technique is carried out with GC instruments in which the injector system has been modified into a pyrolysis chamber.[86, 87] After being loaded into the pyrolysis cell, the sample is heated there to high temperatures at which the contained analytes are thermally decomposed and the formed products are transferred by the mobile phase stream into the column.

Around 1959 early devices for pyrolysis GC were reported, one consisting of a metal loop, which replaced a gas sample loop at the inlet of the packed column.[87] To accomplish thermal sample decomposition, the loop was heated in a bath of Wood's Alloy, while the temperature achieved by this heat source was measured by a thermocouple connected to a pyrometer. This design showed in principle all necessary functional features of modern pyrolysis GC, but it lacked provisions for the accurate adjustment of the instrumental parameters, which are essential for the reproducible sample decomposition and subsequent chromatographic analysis. Parameters crucial to reliable analysis by pyrolysis GC are a well-defined and constant pyrolysis temperature, a precisely controlled heating rate, and a defined duration of the pyrolysis. A gas chromatogram obtained with the pyrolysis GC prototype device mentioned above for a synthetic polymer (applying 500°C for 30 sec in a helium atmosphere) is shown in Figure 11, left panel.

First instrumental improvements of the pyrolysis process were obtained by loading the sample onto an electrically heated metal wire or into a spiral[86, 88], with the temperature being calibrated by the melting points of suitable reference compounds. Alternatively, other devices for accurate temperature adjustment were using ferromagnetic metals which, upon placement into a high frequency inductor coil, were heated exactly to the Curie point and maintained a constant temperature by self-stabilization. Modern commercial instruments regulate and control all crucial operational parameters fully electronically. Equipped with platinum filaments, state-of-the-art pyrolizers can be operated at temperature up to 1400°C (which can be kept constant with an accuracy of 1°C), with flash pyrolysis heating rates up to 1000°C/s, and can be programmed to execute in highly reproducible fashion sophisticated user-defined pyrolysis protocols.

Upon thermally cracking the sample under well-defined conditions, the resulting pattern of chromatographic peaks allows conclusions to be drawn about the kind of the sample by comparison with reference materials and the identification of marker peaks. Coupling MS detection devices to the GC column provides the possibility to gain detailed structural information. A striking advantages of pyrolysis GC over more conventional approaches is that samples can directly be analyzed

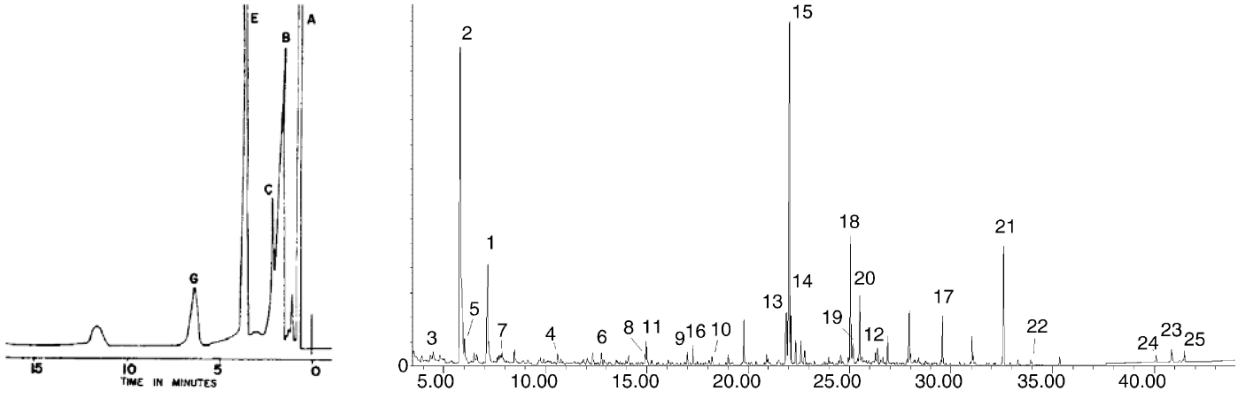


**Figure 11.** Introduction and development of Pyrolysis GC. *Left panel:* Early Pyrolysis GC with packed column published in 1959. Sample: polymethylmetacrylate. Packed column, isothermal 100°C; Pyrolysis at 500°C for 30 sec in He atmosphere. Peak annotation: A, air; B, methanol; C, ethanol; E, methyl acrylate; G, methyl methacrylate. For details, see text and ref. [87]. From ref. [87] with permission. *Right panel:* Pyrolysis capillary GC recorded using a state-of-the-art instrument in 2005. *T*-programmed mode. Detection: TIC of MS. Sample from a painting in "oleoresin" technique. Peak annotation: 23, hop-22(29)-en-3-ol; 24, β-amyrin; 25, α-amyrin; all as TMS esters. For other peaks see ref. [89]. For details see Supplementary Information and ref. [89]. From ref. [89] with permission.

without any time-consuming pretreatment steps, such as dissolution, hydrolysis, derivatization, etc. Moreover, simultaneous derivatization without elaborate pretreatment can be carried out *in situ* by admixing suitable derivatization reagents to the sample in the pyrolysis chamber.

The improvement of pyrolysis GC instrumentation becomes obvious upon comparing the chromatograms in Figure 11, left panel *vs.* right panel.[89] For the latter chromatogram the sample was taken from a painting executed in "oleoresin" technique by the Mexican artist Carmen Lopez, and for which the pyrolysis was carried out using an *in situ* silylation protocol. As the resulting chromatogram is rather shown for comparison, only the peaks of three sample constituents are assigned, *i.e.* those of the pentacyclic $C_{30}H_{50}O$ triterpenols hop-22(29)-en-3-ol, and α- and β-amyren. The results are indicative for a special resin, Mexican copal, as a constituent of the painting medium.

As useful as pyrolysis GC has proven for the analysis of binding media, we wish to place here a word of caution. Despite of the maturity of the method, reproducibility of the results remains an issue, especially when different experimental conditions are applied, and instrumentation supplied from different vendors is used. Moreover, it has been pointed out that the quantitation of specific analytes with pyrolysis GC might be less reliable than that carried out with conventional chromatographic methods. Readers interested in a more in-depth treatise of the contributions of pyrolysis GC to binding media in objects of cultural heritage are directed to ref. [90].

## 9. COLUMN SWITCHING, HEART CUTTING, TWO-DIMENSIONAL GC

When being challenged with highly complex samples, not all compounds of interest may be successfully separated with GC employing a single column. Rather, a number of analyte zones may co-migrate and form overlapping peaks with the consequence that reliable identification and quantitation remain elusive. In this case, application of a column with a different chromatographic retention characteristic might resolve some critical pairs, but still may fail to separate other compounds of interest. A solution to this problem is to combine two columns of low chromatographic similarity in series, via a switching valve interface as introduced by D.R. Deans[91]. This method, referred to as heart cutting or column switching, enables the transfer of a certain fraction of eluate from the first column (1) onto the second column (2), both being equipped with separate detectors. In column (2) this fraction is chromatographed simultaneously with the sample components remaining at column (1). Given that the columns have been selected appropriately in term of complementary selectivity, successful separation (and detection) of the initially unresolved peaks may be achieved.

Certainly, column switching is not limited to only one unresolved peak pair, but may be repeatedly applied to many peak clusters in the same chromatogram. In case that the set of employed columns possess completely different chromatographic properties (*i.e.* are "orthogonal"), they are considered to represent different chromatographic dimensions and the method is named two-dimensional GC. This terminus is taken from two-dimensional gel electrophoresis and thin layer chromatography. The similarity of the chromatographic properties of the stationary phases (and thus the "dimensionality" of the two combined columns) may be established quantitatively using chemometric methodologies[92, 93], and to some extent by comparison of their Rohrschneider-McReynolds indices for the polarity.

To the best of our knowledge, column switching approaches have not yet been applied to the analysis of binding media, although it should have a high potential, especially for the separation of the components in very complex mixtures of different binders in the same sample.

## 10. ON-LINE COUPLING GC-MS

Technically, coupling a gas chromatograph with a mass spectrometer is a nearly ideal combination of two powerful analytical techniques, because in both methods the analytes are present in the gaseous phase, albeit at pressures that differ by about 8 orders of magnitude. First attempts to realize this attractive option of an on-line combination of GC with MS were carried out with packed columns, but were complicated by the large volume of the GC gas flow, which, unsurprisingly, compromised the high vacuum conditions required for MS operation. Solutions to this problem were sought by the design of innovative interfaces to harmonize the mutual flow requirements of the GC and MS module[94-98], respectively, by jet-type or by membrane separators, or by direct open coupling as described in ref. [99]. With the latter interface packed columns with inner diameters of up to 4 mm were successfully coupled with a double focusing MS (see e.g. ref. [100]), though some losses in sensitivity and an impairment of the detection limit of the GC-MS method were resulting.

For analyses of binding media, application of packed column GC-MS coupling emerged in the 1970s, and a

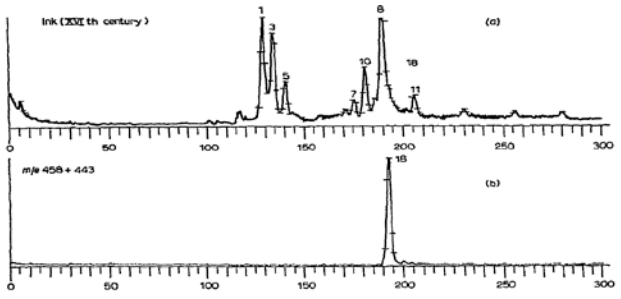


**Figure 12.** One of the first on-line GC-MS for binding media analysis: computer reconstructed plots of (a) TIC of a 16th century ink hydrolysate; (b) mass chromatogram at m/e 458 plus 443 specific for gallic acid (all analytes as TMS derivatives). Packed column, *T*-program; MS scan speed 1 set/decade in cyclic mode, period 4 s. Peak numbering: peaks 1, 3, 5, 7, 8: furanose and pyranose epimers of arabinose and galactose, resp.; peaks 10 and 11, α- and β-glucopyranose, resp.; peak 18, gallic acid. For details see Supplementary Information and ref. [101]. From ref. [101] with permission.

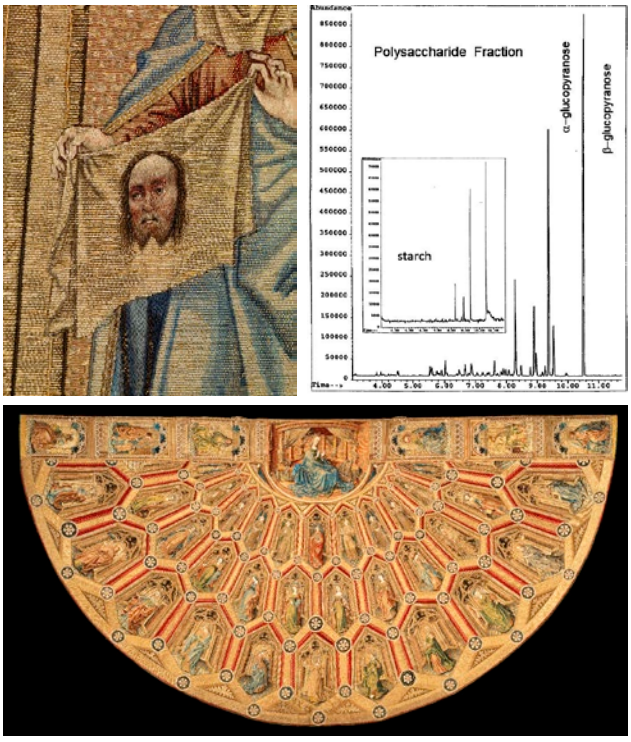


**Figure 13.** GC-MS of the polysaccharide fraction from a sample of the *Cope of the Virgin Mary* (15th century). The Cope measures 330 cm in width and 164 cm in length. Peaks: α- and β-glucopyranose. (TMS derivatives after hydrolysis and concomitant group-separation by an ion exchanger from the proteinaceous fractions). *Insert:* GC-MS of barley starch; same procedure as the for sample of the Cope. Time in min. Peaks recorded from TIC. For details see text, Supplementary information and ref. [102]. From ref. [102] with permission. *Bottom and left picture:* Long shot and detail of the *Cope of the Virgin Mary*. Photographic images by courtesy of KHM-Museumsverband, Vienna, Austria.

chromatogram documenting one of these early efforts is shown in Figure 12. This study was reported in 1977 and aimed at identifying plant gums and gallic acid as possible constituents of an ink sample of a European manuscript on parchment from the 16th century.[101] Prior to analysis, the ink sample was hydrolyzed and the emerging products subsequently silylated. A packed column of 2 mm i.d. was connected to the MS with a single-stage jet-type separator. The chromatograms were reconstructed by summing up the ion current of each scan. In trace (a) the total ion current (TIC) is recorded, and arabinose, galactose and glucose are identified. Being isobaric, the epimers of glucopyranose could not be differentiated by their mass spectra, but plausibly identified based on their chromatographic retention order. The extracted dual ion monitoring trace depicted in trace (b) with two m/e values specific for silylated gallic acid allowed its reliable identification. Based on these results, the authors concluded that the investigated manuscript was written using ferro-gallic ink containing gum Arabic as a binder.

The mismatch between the gas flow volumes at the outlet of the packed GC columns and the inlet into the MS could largely be avoided by the use of capillary columns. Due to the lower gas volume emerging from the capillary, the column outlet could directly be coupled via a heated transfer line to the MS without impairing its vacuum, enabling the introduction of the entire flow volume into the ion source of the MS. Moreover, for routine analyses the sector MS instruments were replaced by the less expensive quadrupole mass spectrometers with their much faster scan rate, and with their very user-friendly operability. Evidently, the combined benefits of a high level of technical maturity, and the ready availability at reasonable costs have made GC-quadrupole MS the most popular analytical method in laboratories devoted to the investigation of organic materials in museum objects.

In Figure 13 the result of directly coupled capillary GC-quadrupole MS for the analysis of a polysaccharide sample is shown[102], processed after hydrolysis and silylation. The sample was taken from the *pluviale*, known as the *Cope of the Virgin Mary*, a liturgical vestment of the Order of the Golden Fleece, housed in the Imperial and Ecclesiastical Treasury in Vienna, Austria (see Figure 13). It was fabricated between 1425 and 1440 in Burgundy and most likely designed by the Master of Flémalle (highly probably identified with Robert Campin, c. 1375 - 1444). The vestment has been executed using two embroidery techniques, *viz.* in needle painting and in *or nué*, a lazur embroidery technique. The

Cope measures 330 cm in width and 164 cm in length. The background structure of the Cope consists of several layers of textile materials stitched together. Originally, these textiles may have been partially conglutinated, as small white round-shaped scales of about one square millimeter in size were found between the layers, probably applied as a gluing agent. For restoration and conservation purposes, it was of interest to identify the kind of this gluing material. For plausibility reasons, an analytic strategy capable of differentiate between animal and plant glues was chosen for this project.

Prior to GC-MS analysis, a mild hydrolysis protocol suited for a potentially present mixture of proteinaceous and polysaccharide binders was employed, using a cation exchange resin in the $H^+$-form as a hydrolysis reagent.[103] Importantly, this particular procedure provides additional benefits as it permits the separation of the protein hydrolysate from polysaccharide-based materials and prevents these classes of compounds from undergoing condensation reaction with each other. After the group-specific separation of the two binder classes, hydrolysis of the polysaccharide fraction was completed, and both hydrolysate solutions were subjected to appropriate silylation protocols. GC-MS analysis of the amino acid fraction did not provide any products consistent with amino acids, thus the presence of proteinaceous binder could be safely ruled out. However, in GC-MS analysis of the derivatized saccharide fraction two main peaks were detected, both of which were identified by MS being epimeric glucopyranoses; yet efforts towards unambiguous stereochemical assignment of the individual epimers proved difficult due to their almost identical mass spectra. However, as the α-epimer has a smaller retention factor than the β-epimer, the two peaks could be reliably assigned (see also ref. [101]). Given the fact that no other saccharides were detected, the unknown material employed as a gluing material in the vestment was positively identified as starch (see insert in Fig. 13). Subsequently, this conclusion was independently confirmed by consistent results obtained by analyzing the other possible polysaccharides as reference samples under identical conditions.

It should be pointed out that advanced protocols for group-specific isolation of lipids, animal and plant glues, waxes and resins prior to GC-MS analysis are described in refs. [104, 105]. These procedures involve mainly liquid-solid, solid phase, separation sorbent-tip, and clean-up by ion exchange extraction steps, respectively.

Historically, only a limited number of investigation of organic matter in museum objects with GC-MS were published before the turn of the millennium, but the acceptance of this technique as an enabling tool for the

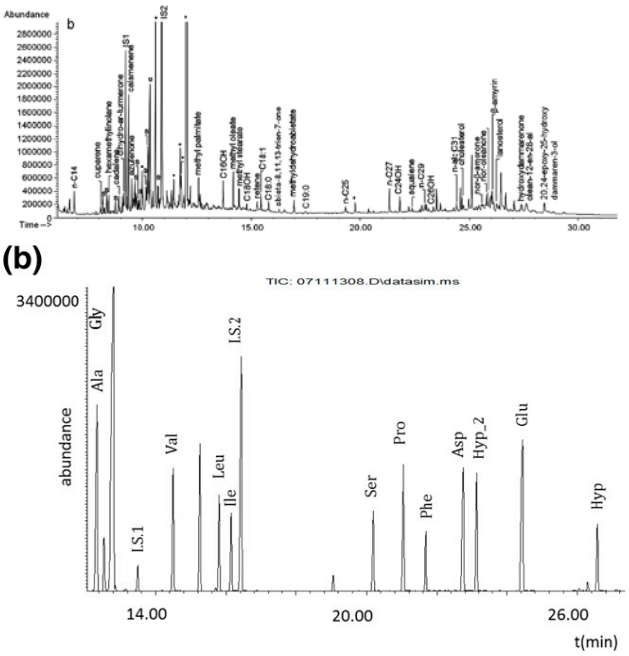


**Figure 14.** GC-MS of binding media in objects from Antiquity and Renaissance. *Top panel:* TIC chromatogram of sample of embalming materials in Egyptian skulls (neutral fraction from extraction). Sample from the archaeological collection, Natural History Museum, Florence, Italy. According analytes as TMS-derivatives. Column, fused silica capillary; *T*-program, MS: single quadrupole. For details see Supplementary Information and ref. [107]. From ref. [107] with permission. *Bottom panel:* capillary GC-MS of amino acid fraction of egg tempera sample from polyptych "Annunciation and Saints" (ca. 1385), Giovanni del Biondo (ca. 1356–1398), Galleria dell'Accademia, Florence, Italy; sample pretreatment and experimental conditions see ref. [104]. Taken from ref. [108] and modified.

analysis of organic matter in museum objects has significantly increased over the last two decades. This is manifested by the growing number of groups being active in the field as well as the ever-increasing number of papers published on the topic. The objects investigated range from archeological finds and artifacts from antiquity[106, 107] (see Figure 14, top panel) over those from Middle Ages[108] (Figure 14, bottom panel) up to contemporary art works.

As an addendum we mention that the GC-MS protocols developed for the determination of proteinaceous binders are exceptionally capable of distinguishing between classes of parent proteins, *i.e.* egg, casein or collagen, but unsuitable for identifying the species of the animal from which this protein originates. This question can be addressed using bioanalytical methods, originally developed for clinical diagnostics, such as enzyme linked immunosorbent assay (ELISA) or by modern proteomics protocols. ELISA methodology is based on selec-

tive binding of the protein of interest to a target-specific antibody. Proteomics protocols, recently adapted to binding media studies[109], involve the enzymatic digestion of the protein sample of interest using appropriate hydrolytic enzymes (typically trypsin), separation and identification of the formed peptides via LC-MS techniques, and finally matching of the observed peptides with appropriate databases for identification of the original protein(s).

The suitability of ELISA and proteomics protocols for the species-specific identification of proteins in the binding media has been recently demonstrated for Egypt-Romano Portraits dating to 180-200 A.D., for which cow hides could be established as protein source. [110] ELISA and proteomics assay were also employed, together with a number of spectrometric techniques, for the identification of the proteinaceous material found in late medieval mortars, and their merits and limitations were critically discussed.[111] A study into the proteinaceous binders present in the Giant Buddha statues of Bāmiyān in Afghanistan (largely demolished in 2011) was carried out using a combination of GC-MS and proteomics techniques. In this case, egg tempera was found in the original paint layer, while cow and goat milk was detected in historical overpaintings.[112]

Certainly, ELISA and proteomics protocols are useful to complement the knowledge accessible with established GC-MS methods, allowing identification of species-specific sources of proteinaceous binders. Currently, however, these methods are rarely applied. Reasons for this reluctance may be the need for specialized instrumentation and the high level of expertise required; and the question whether or not the high costs associated with these techniques are justifiable by additional scientific information potentially gained.

To round off the discussion about the role of GC-MS in binding media studies, we wish to reflect on a very particular coupling technique evaluated at the end of the 1980s by one of the authors (E.K.). In co-operation with a major instrument vendor, the opportunity arose to test an innovative GC-FTIR-MS prototype. In this configuration, a low-volume IR cell was directly connected to the outlet of the capillary column, allowing the non-destructive detection of GC effluents prior to transfer to the quadrupole MS. This GC-FTIR-MS combination was successfully used for the identification of some non-resinous compounds detected in the chromatograms of samples of anatomic wax models from the 18$^{th}$ century. [60] However, there may have been little general interest in GC-FTIR-MS at this time, as the instrument never made it to the market. Thus, our scientific exploits may well have been the first and the last application of GC-FTIR-MS to the analysis of organic binding media in museum objects.

## 11. CONCLUSIONS

The development of GC has been a long journey, which started in the late 1800s with the pursuit of methods for preparative gas separation and ultimately led to the establishment of one of the most versatile and sensitive modern analytical separation techniques. It needs to be emphasized that the evolvement of GC was made possible through the ingenious contributions of many outstanding scientists both in the fields of theory and applied research. Important historic milestones in the development of GC are numerous; these involve i) the early efforts to adapt known adsorption liquid solid chromatographic concepts to gas solid separations; ii) the subsequent transition from solid adsorptive stationary phase to solid-supported liquid stationary phases, dramatically expanding the scope of interactions forces for tuning separation selectivity; iii) the establishment of a thorough understanding of the parameters that control retention and dispersion in chromatographic columns, and the theoretical understanding of how these contributions impact the overall achievable separation performance; iv) the following theory-guided transition from packed columns to open tubular capillary columns, obviating the performance-degrading particle beds and allowing for tremendous improvements in separation efficiency by using vastly enhanced column lengths; v) and the parallel occurring improvements in chromatographic materials and instrumentation, such as inert column materials, thermally highly stable stationary phases, improved injection systems and highly sensitive detectors, and, finally, vi) the optimized coupling of GC with mass spectrometric detection devices.

The potential of GC for the analysis of binding media has been recognized as early as in the mid-1960s, and has found ever-increasing appreciation as the technology approached maturity. State-of-the-art GC instruments offer the benefits of low sample requirements, high sensitivity and separation selectivity, and straightforward analyte identification when coupled with mass spectrometric detection devices. Special GC techniques, such as pyrolysis GC, can be employed to provide valuable insights into the compositions of samples difficult to characterize by other techniques, such as high molecular mass compounds and polymers. Given these advantages, GC is currently appreciated as a standard tool in the field, and routinely employed for the characterization of all important media classes.

Other techniques are increasingly added to the repertoire of analytical tools employed binding media analysis; especially worth mentioning are dedicated Raman and IR spectrometric techniques, which offer the benefit of being fully conservative. Also, new chromatographic methods, in particular those involving LC-MS proteomics methodology, are finding recognition as tools for characterization of protein-based binding media to identify not only the class but also the species-specific source. For the same purpose, also bioanalytical approaches based on immunoaffinity binding, such as ELISA are employed.

While the advent of these new methods will certainly enhance the information accessible through binding media analysis, it is unlikely that they will replace GC as the prime enabling technique. GC, while being a micro-destructive analytical method, is uniquely suited for binding media analysis with regard to its low sample requirements, the broad scope of materials that can be analyzed, its inherently high sensitivity, and its perfect compatibility with mass spectrometric devices. Also, there is a uniquely rich body of expertise and experience with GC in binding media analysis that has been compiled over five decades, which facilitates the interpretation of results, and is invaluable for guiding the design and execution of new studies.

Future progress in the field of GC-based binding media analysis may involve improvements in detection sensitivity by new generations of mass spectrometric devices, which is anticipated to further reduce sample amount requirements. Also, future binding media analysis may potentially profit from the implementation of the emerging comprehensive two-dimensional GC technology using stationary phases with different retention characteristics, resulting in enhanced information outputs to better justify the use of precious and unique sample materials.

Arguably, GC will remain a technique of central importance in the field of binding media analytics in cultural heritage research and preservation.

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## COMPETING INTERESTS

The authors declare no competing interests.

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