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The Potential of Pyrolysis-Gas Chromatography for the Pattern Individualization of Macromolecular Materials

In an investigation almost any article or material may come to the attention of the forensic scientist as physical evidence. Frequently he is asked to test the existence of an association or common origin between the questioned material and some other sample to the exclusion of all (ideally) other similar samples of the same group or class. This process or operation is referred to as individualization by forensic scientists. If the sample is an object which has certain morphological peculiarities or physical markings, these may be compared with those on an item that is thought to have been associated with the questioned one to test this hypothesis. If, however, the item or material lacks these markings, it may be necessary to consider compositional differences. The approach used to study such differences will vary depending on the general type of material being analyzed.

Accurate methods for measuring physical properties such as density and refractive index are often superior to many chemical techniques for detecting small compositional differences with stable types of physical evidence such as glass [*1*, *2*]. This situation is in part a result of limitations in the quantitative accuracy and reproducibility of many present-day chemical and instrumental methods, especially when the limits of detection are being approached. Future improvements in analytical instrumentation should reduce the forensic importance of physical property measurements in making discriminations of this type. For materials with suitable elemental compositions such as metals, alloys, glass, minerals, soils, and inorganic chemicals, techniques such as emission spectrography, X-ray fluorescence, spark source mass spectrometry (SSMS), or neutron activation analysis (NAA) would be indicated. A refined atomic absorption (AA) technique with a real-time multiple element capability might also be useful for individualizations of this type. The forensic individualization of glass has been studied using NAA [*3*] and SSMS [*4*].

The more sensitive of the instrumental approaches might be appropriate for determining the trace element distributions of certain predominantly organic substances if the numbers and concentrations of the elements of interest were sufficient. The elemental composition of hair, for example, has been studied using emission spectrography [*5*], AA [*6*], X-ray fluorescence [*7*], SSMS [*8*], and particularly extensively with NAA [*9–11*]. The work with hair has been summarized by Morton [*12*]. One of the shortcomings of

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these methods with organic materials such as hair is their inability to deal reliably and precisely with small amounts of these trace elements in samples of limited size, such as those encountered in forensic investigations. Trace element distributions at the parts per million (ppm) level are difficult to study in a sample, the total mass of which may be on the order of a few micrograms. In such a case the ability to accurately measure subnanogram amounts is necessary.

For volatile substances such as solvents, gas chromatography would probably be best for presenting a sensitive display of the trace organic composition. Chemical or field ionization techniques used with mass spectrometry are other more recent alternatives.

The method of choice for obtaining an individualization based on organic composition for small quantities of macromolecular materials would be pyrolysis-gas chromatography (PGC). This technique has been shown to be sensitive to differences in composition for numerous synthetic polymers [*13*], and has even been employed to successfully demonstrate differences in crosslinking for polymers of the same composition [*14*]. Oyama [*15*, *16*] and Reiner [*17*, *18*] have pyrolyzed different strains of bacteria and identified them by their characteristic pyrograms.

PGC has been employed by forensic scientists for more than a decade. It has been exploited very satisfactorily for the identification of substances of forensic interest such as drugs, including barbiturates [*19*], alkaloids [*20*], and phenothiazines [*21*] and for polymeric materials including plastic resins [*22*], fibers [*23*, *24*] and paint [*25*]. Attempts at individualizing paints [*26*] and hair [*27*, *28*] have met with less success although they have shown promise. For individualization, the highest degree of discrimination between closely related substances is essential. To attain this degree of discrimination high sensitivity and high resolution are both necessary, as has been pointed out in connection with attempts to individualize paint [*26*] and hair [*27*, *28*] by PGC. To meet both of these conditions simultaneously and to satisfy the conditions for optimizing the pyrolysis parameters poses some formidable problems, which will be discussed below.

Theory—Instrumental Considerations

Chromatographic Conditions

The sensitivity of any gas chromatographic analysis depends to a large degree on the device employed to detect the column effluent, the most sensitive of these being the ionization detectors. The flame ionization detector (FID) is the least selective of this type. It responds to a wide variety of classes of organic compounds and is therefore the most generally used ionization detector. Any individualization of macromolecular materials based on PGC would probably employ flame ionization detection over any alternative systems currently available because of the wide variety of substances likely to be produced in pyrolysis. Other more selective detector systems might be used as adjuncts to this. Sensitivity can be limited by the column liquid phase used. If the liquid phase bleeds off the column, it can make it necessary to reduce the sensitivity. This is especially true where temperature programming is employed.

A large number of products belonging to many chemical classes can be expected to result from the pyrolysis of complex macromolecular materials. This places stringent separation requirements on the chromatographic system. Successful separation of complex mixtures of compounds in a wide range of molecular weights would dictate the use of temperature programming in conjunction with a high resolution column. Packed columns are those most commonly chosen for gas chromatographic separations. However, the resolution of these columns is inherently inferior to that attainable with open tubular

(capillary) columns developed by Golay [*29*]. Ideally, a capillary column should be utilized to meet the resolution requirements necessary for a successful individualization. These columns, however, have several disadvantages. For this reason Levy [*26*] has suggested that support-coated, open-tubular (SCOT) columns be used for individualization work by PGC. The design of these columns is a compromise. The central portion of the bore is open as it is with Golay-type columns, but the wall is covered with a granular solid support similar to the material used in conventional packed columns. The liquid phase is coated on this support material. A larger quantity of liquid phase can be used with these columns than is possible with wall-coated, open-tubular columns in which only a thin film of the liquid is retained on the inside of the bore. For this reason SCOT columns are capable of handling larger samples without overloading the liquid phase. The resolution of these columns is intermediate between that of capillary columns and packed columns. The author's experience with PGC applied to hair suggests that the highest attainable resolution is necessary in order to realize the goal of a true PGC individualization [*27*]. This dictates the use of capillary columns.

Some difficulties must be overcome before capillary columns can be adapted to this work. The conventional method of sample introduction with an open-tubular column is by sample splitting, whereby the majority of the sample is discarded in order to keep the volume of sample introduced small. The split ratios used are often as high as 300:1. The idea of discarding more than 99 percent of the sample would be untenable in many forensic science applications because of the limited amount of sample normally available. As will be discussed later there are also theoretical considerations concerned with pyrolysis that make this unacceptable. De Forest [*27*] explored an alternate means of sampling with capillary columns that had certain advantages for use with pyrolyzates including the elimination of sample splitting. A cryogenically cooled capillary pre-column was used with a bypass which allowed a large volume of dilute pyrolyzate to be concentrated in the pre-column prior to analysis. Because of technical difficulties (for example, excessive dead volume), this approach was not as successful as had been hoped. Further mechanical refinements should make this or a similar device workable.

Pyrolysis Conditions

Numerous types of pyrolysis devices have been described in the literature. These have been discussed and categorized in a review by Levy [*30*]. For samples which can be put into solution, certain types of pyrolyzers where the sample can be applied in the form of a thin film are clearly superior [*30*, *31*]. For insoluble macromolecular materials the choice is more involved. Ideally, the sample should be heated extremely rapidly to a predetermined temperature and, in addition, the pyrolysis products should be removed from the reaction zone as they are formed to minimize the occurrence of secondary reactions. The criterion of a rapid heating rate can be met in different ways. The sample can be pulse heated rapidly by utilizing a filament in conjunction with special circuitry [*32*], or by using a radio frequency field to heat a selected ferromagnetic material to its Curie point [*33*]. As an alternative a laser [*34*] can be employed. A simpler device with certain advantages for insoluble solid samples (for example, hairs, fibers, paint, etc) employs a sample area (furnace) which is held at a carefully controlled temperature. The sample is heated nearly instantaneously when it is rapidly introduced into the high temperature zone. In one commercially available pyrolysis unit of this type (Hamilton Multipurpose Sampling System) sample introduction is accomplished by gravity [*27*]. This unit and the laser type would seem to have the greatest applicability to irregular samples of intractable macromolecular materials. The rapid removal of pyrolysis products from the reaction zone

depends on the method of introducing the sample to the chromatographic column as well as on the characteristics of the pyrolysis device itself. The system with the capillary precolumn [*27*] described above was designed to provide rapid removal of the pyrolyzate and to allow sampling without sample splitting. This system makes it possible to use small samples, which is an important consideration in forensic work. A further advantage is realized due to the fact that small samples are preferable in pyrolysis [*35*]. Small samples are easier to pyrolyze reproducibly because the opportunity for the occurrence of secondary reactions is reduced.

Discussion

In general a useful individualization for forensic purposes requires that a sufficient (often the maximum) amount of information be extracted from as little as a few micrograms of sample. As was pointed out above, to attempt to accomplish this by PGC it is necessary to employ a system that is capable of providing the highest attainable resolution and sensitivity for a large variety of substances in greatly varying concentrations. This implies the use of state-of-the-art advances in GC instrumentation pertaining to separating power and ability to deal with minute samples. However, there seems to have been a shift in emphasis away from strictly analytical refinements in recent years. This is apparently because fairly simple gas chromatographs are capable of handling a host of chemical problems [*36*]. A separation of almost any given pair of substances in a mixture is fairly easy to accomplish with judicious column selection and choice of instrumental parameters (for example, column temperature and flow rate). Simple instruments can handle this type of work using only rudimentary components. Where the sample available is adequate a thermal conductivity detector will suffice. A number of basic inexpensive instruments of this type which are suitable for a variety of routine applications are on the market.

Instrument manufacturers have, in large measure, been focusing on automated analysis and data reduction. Automatic sample injectors and recycling temperature programmers are available with many gas chromatographs. Sophisticated data systems can be obtained which can be programmed to type out the results of an analysis in a report format. Advances of this type offer many exciting possibilities. They do not, however, improve the quality or sophistication of the chromatographic analysis. They merely provide a savings in labor and take the emphasis off attaining the maximum sensitivity and resolution which were the aims of early gas chromatographers.

Advances in detector design have centered around selective detectors rather than those which respond to a wide variety of compounds. Detectors of this latter type which are sensitive to a broad spectrum of compounds (for example, FID) are little more sensitive than they were a decade ago, although it must be admitted that problems such as column bleed, not intrinsic detector performance, are often the limiting factors in attaining high sensitivity. New, more stable liquid phases are now becoming available which should serve to reduce this limitation.

Rudzitis [*37*] has pointed to the desirability of identifying peaks in a pyrogram for purposes of individualization. This might be a useful approach at the research stage of individualizing macromolecular materials by PGC. It is doubtful, however, that this approach would offer any advantage when the method is used routinely for the practical laboratory individualization of physical evidence. The constraints placed on the method necessitated by peak identification could severely hinder the individualization for a number of reasons. Under the best of conditions it might only be possible to identify the larger peaks. However, the smaller peaks, as suggested in the work with hair, would probably be the ones most important for individualization because many of the large peaks would

be expected to be common to all samples from the same general group or class [*28*]. The use of more complex equipment to accomplish this limited goal of peak identification would be difficult to justify in the routine application of PGC as an individualization tool.

Standardization of technique would be desirable if PGC were to be used for interlaboratory identifications of samples of interest in forensic investigations. Strict standardization would not be as important for intralaboratory individualizations where the patterns of known and questioned pyrograms could simply be compared directly. Refinements of other factors discussed earlier would be of prime importance here so that small differences could be detected if present. A knowledge of the variation of pyrolysis patterns in the population or class of evidence under consideration would also be essential. The employment of PGC in individualization studies should not await developments which allow for better interlaboratory standardization. It is doubtful that the degree of standardization required for interlaboratory identifications is readily attainable [*38*]. The main difficulty would appear to lie with the standardization of the gas chromatographic separation. Complex column variables preclude rigid standardization of even the simplest gas chromatographic separations. Even if parameters such as flow rate, column temperature, and programming rate are held constant, small intrinsic differences between columns are likely to remain. In addition, columns are likely to change with time and usage. Because of their variable nature, gas chromatographic curves do not have the same interlaboratory value as infrared absorption curves or mass spectra. This degree of standardization is not required for an individualization. It would, however, be essential for identification of substances where reliance on data from other laboratories is necessary. The pyrolysis aspect of a PGC procedure is far easier to standardize provided a pyrolysis device of the same type is employed [*30*].

At the present time it would appear that the furnace-type pyrolysis system would be best for use with intractable materials such as hair. The filament and Curie point types are designed for use with samples which can be dissolved and coated, in the form of a thin film, directly on the filament or ferromagnetic material. Many samples of criminalistic interest are not amenable to such solution-coating techniques. An additional problem is presented by the catalytic nature of these metallic and metal oxide surfaces [*39*]. Such surfaces could change with time and usage, complicating the situation. The laser-type pyrolysis system seems to offer certain advantages especially with regard to instantaneous heating of the sample. However, the heating rate might be expected to vary with the sample geometry and absorbance. Difficulties have been encountered in transferring the laser energy to the sample. Some workers have found it necessary to grind the samples and mix them with powdered graphite to obtain an efficient coupling of the laser energy to the sample [*40*]. The color of the sample, the amount of graphite powder used, and the diameter of the incident beam have also been shown to have an effect on the reproducibility of the technique [*41*, *42*].

In light of the failure to achieve an individualization by PGC to date, it is instructive to review and consider the status of alternate approaches. Other techniques which have been applied to the problem of individualizing macromolecular materials have met with limited success at best. All have fallen short of achieving a true individualization. The most notable of these has been the extensive work with hair using NAA. The work with large samples or bundles of hair has been encouraging, but it is not yet possible to approach an individualization based on the gamma-ray spectrum of a single irradiated hair. The work reported by Yurachek et al [*8*] with SSMS applied to hair was a pilot study with reference to forensic applications. They used gram and milligram quantities of ashed hair from a very limited number of subjects. It is questionable at present whether

this technique offers adequate sensitivity for determining trace element distributions in macromolecular materials of forensic interest. These workers found it necessary to ash the samples prior to SSMS analysis because without ashing a large number of organic fragments were produced. These complicated the spectra and masked the lines of the trace elements present. Thus, these researchers intentionally destroyed the potential information contained in the organic fragmentation spectra. These data could prove to be very useful for individualization. The possibility of using pyrolysis mass spectrometry (PMS) for this purpose has been suggested [*27*]. A preliminary investigation carried out with the cooperation of an instrument manufacturer has shown promise for the forensic individualization of hair and paint by high resolution PMS [*43*]. This technique is worthy of detailed exploration with respect to the individualization of intractable types of organic material. The fragmentation caused by electron impact combined with the thermal fragmentation taking place during pyrolysis could result in patterns that are unnecessarily complex. For this reason a mass spectrometric technique that does not involve electron impact ionization (that is, chemical or field ionization techniques) might be more useful with these types of samples.

Conclusions

It does not seem realistic to expect that separate specialized procedures and dedicated equipment will be developed to meet the needs of individualizing each type of the myriads of materials that may appear as physical evidence in criminal or civil cases. For this reason it is necessary to explore approaches which can be applied to a broad class of evidential materials. One of these approaches might be one which could be applied to certain macromolecular materials as a group.

At the present time it appears that a highly refined PGC procedure offers the best potential approach for the problem of individualizing macromolecular materials for forensic investigations. On the basis of previous work it is possible to specify the minimum equipment that will probably be necessary for accomplishing this. The equipment would include a cryogenically programmable gas chromatograph and flame ionization detection, coupled with stable, highly sensitive electrometers. Capillary columns, modified to allow sampling of large volumes of dilute pyrolyzates, will be necessary to adequately resolve the large number of resulting pyrolysis products. For visual comparison of curves a logarithmic display of several decades of signal would be useful [*44*]. At the research stage of the development of PGC method for this individualization problem an electronic data system with appropriate software for use in comparing curves would be especially helpful. A sophisticated PGC system could be applied to the individualization of many types of macromolecular evidential materials and, in addition, could be used for the identification of plastics, fibers, paint vehicles, and numerous other similar materials of forensic importance.

Summary

It is doubtful that a true individualization of physical evidence based solely on differences in chemical composition has been achieved to date. The need for more research in this area is indicated. The potential of pyrolysis-gas chromatography for attaining this type of individualization with macromolecular materials appears to be good, but only with sophisticated equipment and a highly refined technique. The necessary refinements of the PGC procedure have been outlined and discussed here.

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