MASS SPECTROMETRIC INVESTIGATION OF THE AGING PROCESSES OF BALLPOINT INK FOR THE EXAMINATION OF QUESTIONED DOCUMENTS

INAUGURAL DISSERTATION
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Zusammenfassung

In den forensischen Wissenschaften ist die Bestimmung des Alters einer Kugelschreibereintragung (z.B. Kugelschreiberstrich) ein wichtiges Kriterium bei der Echtheitsprüfung von Dokumenten. Seit Beginn dieser Untersuchungen ist die Altersbestimmung eine wichtige und meist unbeantwortet gebliebene Frage geblieben. Die forensischen Wissenschaftler streiten sich über die Möglichkeit und die Validierung von Datierungsmethoden. Diese Kontroverse ist der Angangspunkt dieser Arbeit gewesen. Kugelschreiberpasten bestehen aus drei Hauptkomponenten: Lösungsmitteln (50%), Farbstoffen (25%) und Harzen (25%). Nach dem Auftragen der Pasten auf Papier findet mit der Zeit eine qualitative und quantitative Veränderung der Zusammensetzung der Kugelschreibereintragung statt. Während Farbstoffe ausbleichen, d.h. vor allem photochemisch abgebaut werden, wird das Alterungsverhalten der Lösungsmittel durch den Verdampfungs- und Diffusionsprozess charakterisiert. In der vorgestellten Arbeit, sind die Alterungsprozesse von Farbstoffen und Lösungsmitteln mittels moderner massenspektrometrischen Methoden und deren möglichen Anwendung zur Altersbestimmung von Kugelschreibertinte untersucht worden.

Zwei Hauptmethoden, Laser-Desorptions-/Ionisations-Massenspektrometrie (LDI-MS) für die Farbstoffen und Gas-Chromatographie-Massenspektrometrie (GC/MS) für die Lösungsmittel, sind für diesen Zweck methodisch weiterentwickelt und validiert worden. Die Alterungsprozesse von Referenzsubstanzen sind unter verschiedenen Bedingungen (Licht, Hitze, Feuchtigkeit) untersucht worden. Dieselben Prozesse sind dann für die Farbstoffen und Lösungsmittel auf der Papiermatrix verfolgt worden. Dazu wurden über mehrere Monate hinweg Eintragungen von mehreren Kugelschreibern dem normalen Sonnenlicht

ausgesetzt und mit im Dunkeln gelagerten Proben verglichen. Dafür wurden einerseits kleine Stücke aus dem Papier ausgeschnitten und auf einem Probenteller befestigt, um dann LDI-MS Messungen direkt von den Tinten auf dem Papier durchzuführen. Andererseits wurden Kugelschreiberstriche für die GC-MS-Analyse aus dem Papier ausgeschnitten und anschließend extrahiert. Außerdem ist Ortaufgelöste LDI-MS getestet worden um die räumliche chemische Verteilung von Tinte auf dem Papier zu bestimmen. Die Reihenfolge zweier sich kreuzender Tintenlinien konnte auf diese Weise zur Echtheitsbestimmung des Dokumentes untersucht werden.

Ein typischer Abbau von Kugelschreiberfarbstoffen ist charakterisiert durch den Verlust von CH_2-Gruppen (siehe Abbildung 1). Die Kugelschreiberpasten enthalten unter anderem sehr häufig Methylviolett (87% der 31 untersuchten Kugelschreiber).

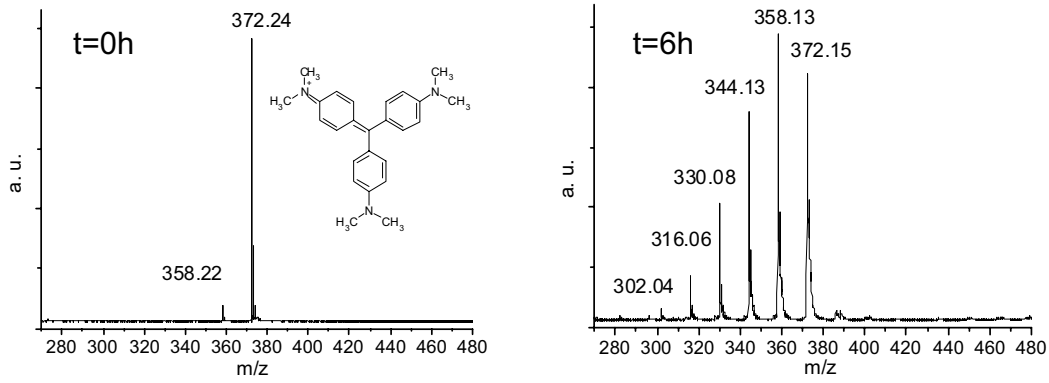

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Abbildung 1 Matrix-Assistierte LDI-MS Spektren des Farbstoffes Methylviolett **(**M^+=372.2 u) in Ethanol gelöst bevor und nachdem er sechs Stunden mit Licht bestrahlt worden ist. Fünf von sechs Abbauprodukten sind im Spektrum sichtbar (Δ=14 u).

Mittels der Beschreibung der relativen Fläche eines Signales (Relative Peak Area, RPA), wobei A_i die Fläche der Signale bei m/z = i und A_{tot} die gesamte Fläche aller Signale der Farbstoffe (Molekul-Ion und Abbauprodukte) ist:

$$RPA_i = \frac{A_i}{A_{tot}} \cdot 100$$

war es möglich, Alterungskurven für den Farbstoffabbau als Funktion der Zeit zu erzeugen. Die RPA-Werte der verschiedenen Signale werden kleiner mit dem Abbau der Farbstoffe oder größer mit der Produktion der Abbauprodukte. Das Alterungsverhalten der Lösungsmittel wird durch den Verdampfungs- und Diffusionsprozess charakterisiert (siehe Abbildung 2). Die Kugelschreiberpasten enthalten unter anderem sehr häufig Phenoxyethanol (94 % der 31 untersuchten Kugelschreiber).

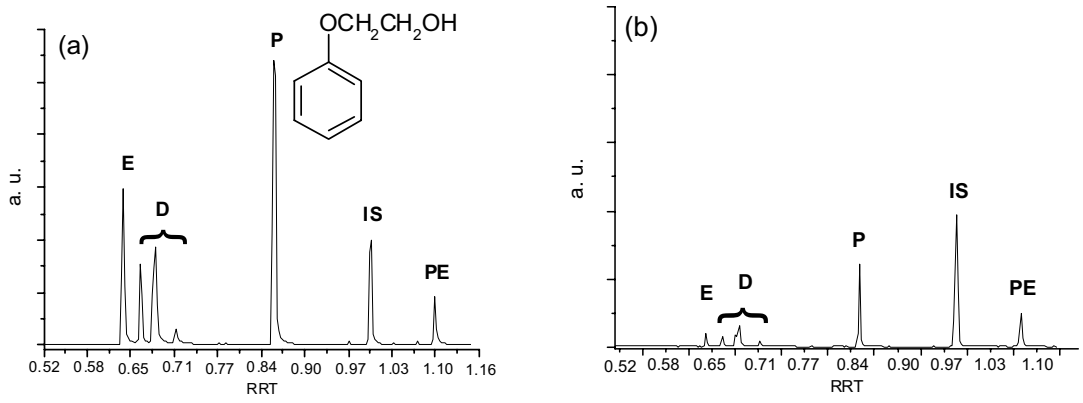


Abbildung 2 GC-MS-Chromatogramm von einem Kugelschreiberextrakt (a) bei der Zeit t = 0, (b) bei der Zeit t = 10 Tage nach der Tintenauftragung auf dem Papier. Die X-Achse stellt die relative Retentionszeit (RRT) zu dem internen Standard (IS) dar. Die Kugelschreibereintragung enthält vier Lösungsmittel: Ethoxyethoxyethanol (E), Diproylenglycol (D), Phenoxyethanol (P, Strukturformel) und Phenoxyethoxyethanol (PE).

Bei Auftragen der Lösungsmittelkonzentration, bestimmt mittels Eichkurven, mit der Funktion der Zeit war es möglich, Alterungskurven der Lösungsmittel zu erzeugen. Die Lösungsmittelkonzentration in Kugelschreiberstrichen sinkt sehr schnell gleich nach dem Auftragen (exponentieller Abfall). Die Ergebnisse über Alterungsverhalten von Farbstoffen und Strichen von Kugelschreibern während einiger Jahre haben viele Faktoren offengelegt, die den Alterungsprozess beeinflussen. Zwei Hauptgruppen sind identifiziert worden: Die ursprüngliche Zusammensetzung der verwendeten Kugelschreiberpaste und die Lagerungsbedingungen des Dokumentes (Abbildungen 3 und 4).

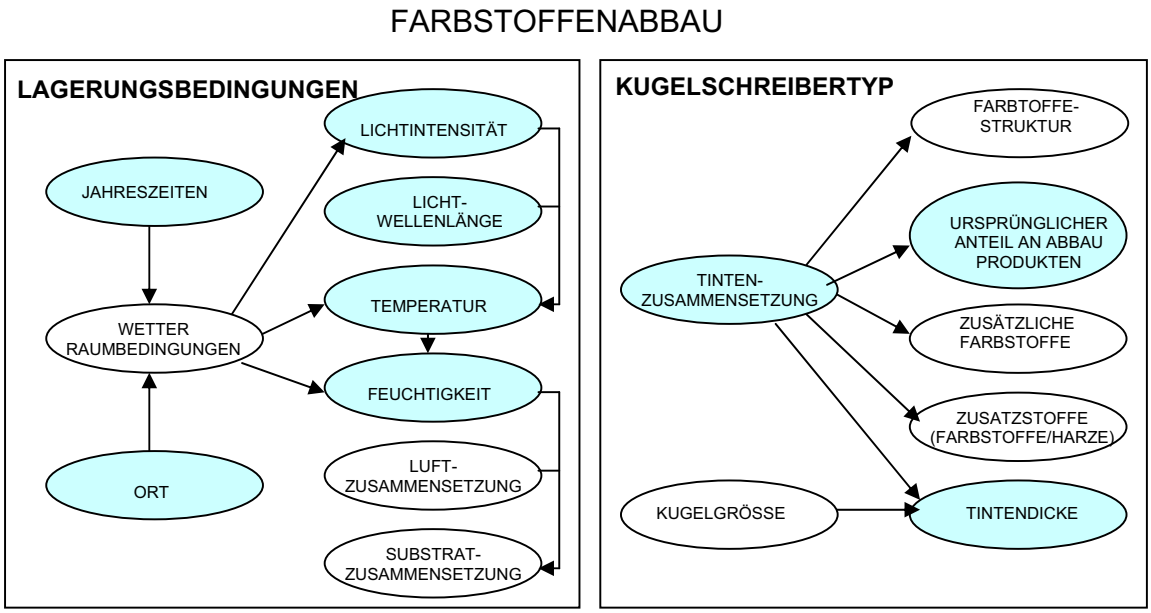


Abbildung 3 Die Einflussfaktoren zum Farbstoffabbau lassen sich in zwei Hauptgruppen unterteilen: Die Lagerungsbedingungen (links) und den Kugelschreibertyp bzw. die Tintenzusammensetzung (rechts). In Blau makiert sind die Faktoren, die in dieser Arbeit untersucht worden sind.

LÖSUNGSMITTELTROCKNUNG

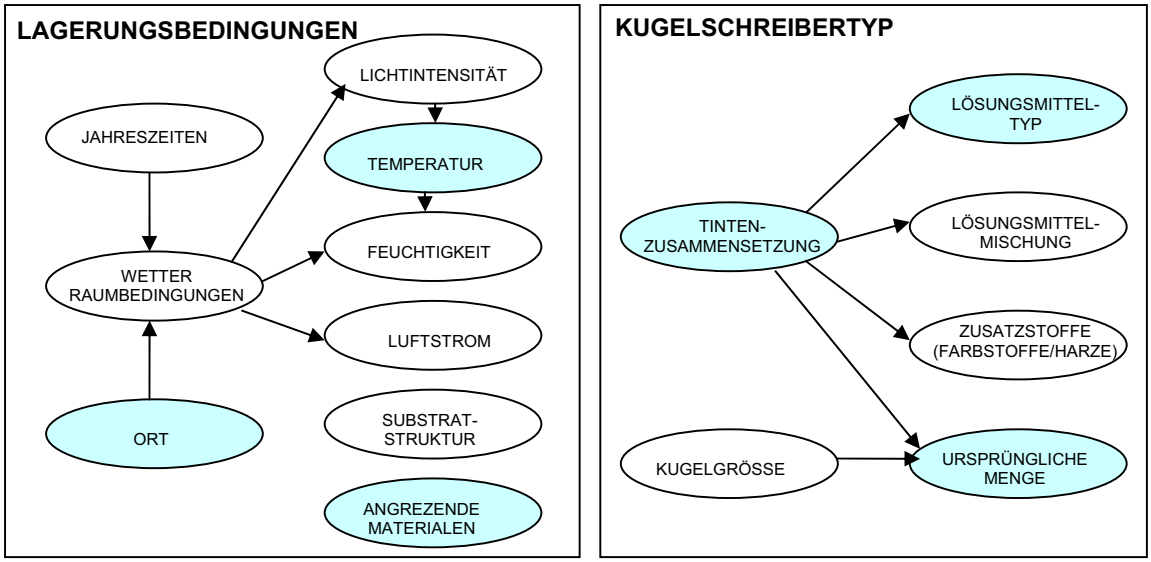


Abbildung 4 Die Einflussfaktoren zur Lösungsmitteltrocknung lassen sich in zwei Hauptgruppen unterteilen: Die Lagerungsbedingungen (links) und den Kugelschreibertyp bzw. die Tintenzusammensetzung (rechts). In Blau makiert sind die Faktoren, die in dieser Arbeit untersucht worden sind.

Um das Altern einer Kugelschreibereintragung zu bestimmen, sollten gute Kenntnisse über alle vorgestellten Faktoren und über deren genaueren Einfluss vorhanden sein. Leider werden in einem echten forensischen Fall diese Informationen selten mit dokumentiert. Die Lagerungsbedingungen sowie der benutzte Kugelschreiber sind meistens unbekannte Größen. Ein Lösungsweg liegt darin, Grenzwerte zu bestimmen, so dass in allen möglichen Fällen die Ergebnisse für eine frische Eintragung immer unterhalb diesen Grenze (z.B. weniger als zwei Monate) bleiben und für eine alte Eintragung immer über einer anderen Grenze (z.B. mehr als einem Jahr) sich befinden. Genügend Daten um alle auftretenden Fälle abzudecken liegen aber zurzeit noch nicht vor. In dieser Arbeit sind massenspektrometrische Methoden entwickelt und validiert worden, um das Alterungsverhalten von Kugelschreiberfarbstoffen und –lösungsmitteln zu

verfolgen. Weiterhin sind Einflussfaktoren zum Alterungsprozess identifiziert worden und die wichtigsten gründlich studiert worden. Es ergibt eine umfangreiche, fundamentale und weitreichend nutzbare Studie der Alterung von Tinte, die bisher nicht existierte. Die Ergebnisse zeigen ausserdem auf welche Probleme ein Gutachter stoßen wird, wenn er eine Altersbestimmung durchführen will. Eine Strategie zur Entwicklung von Datierungsmethoden ist am Ende dieser Arbeit vorgeschlagen. Zuerst sollte eine ausführlische Tintendatenbank aufgebaut werden und zur Verfügung stehen. Gute und detaillierte Kenntiss der Alterungsprozesse aller vorkommenden Komponenten ist erforderlisch. Dazu dient die vorgestellte Arbeit als eine wissenschaftlische und protokollarische Vorgabe. Weitere Werte für bestimmte Alterungsparameter müssen trotzdem bestimmt werden, um eine mögliche Applikation zu entwicklen. Als letzter Schritt muss jede Methode zur Altersbestimmung unbedingt validiert werden.

Le temps est comme un fleuve. Il ne retourne pas à sa source.

Time is like a river. It does not flow back to its source.

Antoine RIVAROL

1 INTRODUCTION

1.1 General context

Ever since paper was invented, documents have taken up a very important place in our society. They play a decisive role in fields such as communication, education, culture, art, sanitation, hygiene, or packaging, and it is almost impossible to imagine life without them. Today, the advances of computer science have put the digital exchange of information into a privileged position in all our societies, yet paper is still a preferred medium in many applications: books, notes, contracts, testaments, receipts, letters, tickets, banknotes, and so on. A great number of transactions can be digitally executed, but in many cases a signature is required for proof of consent. Therefore, frauds committed in connection with documents are not at all uncommon, and in fact represent a very large domain of forensic science called “questioned documents”. In this introductory chapter a general idea of this field and of the problems with which the questioned-document expert is confronted in his routine work will be provided. The difficulties and techniques encountered in the examination of documents, and more particularly those encountered in the present research, will be briefly exposed.

1.2 Questioned Documents

Every day documents are contested: contracts, checks, testaments, medical records, tax invoices, autographs of composers, or pieces of art, to quote just a few. Very important issues depend on the authenticity of a document, and forgeries are probably as ancient as writing. The first case mentioned in the literature goes back to the year 539 [Locard, 1959]. However, it was not before

the 20th century that document examination has taken a scientific direction. Reiss and Bischoff in Lausanne, Osborne in New York, and Brewester in Calcutta were the first experts to write treatises about this field [Osborne, 1910; Brewester, 1932; Locard, 1959]. Although physics and chemistry have introduced a solid scientific dimension, the forensic field of questioned documents is still very complex.

The document expert may have to closely examine a handwritten or printed paragraph, a signature, or an entire document, and determine whether it is genuine or counterfeit. The most frequent questions raised are:

- Who is the author?
- When was the entry produced?

A close comparison of handwritings or typescript may be helpful in answering the first question. In many other cases, paper and ink will have to be compared [Ellen, 1997].

1.2.1 Paper

Human beings are known to have used stone, clay tablets, and many other surfaces for communication or narration during the prehistoric era, witness the drawings found on the walls of some caves or on bones, for example. The word “paper” derives from *papyrus*, a plant growing along the Nile in Egypt, which lent its name to one of the first known writing media created by human beings about 3500 B.C. Papyrus sheets were made by cutting sections of the papyrus plants and pressing them together at right angles, and still survive as scrolls. Since about 1000 B.C., parchment made from animal skins, principally calf, was used in Mideastern Asia. It is probable that paper as we know it today was actually invented in steps, even though Ts’ai Lun is commonly credited in China with the invention of paper in 605 A.C. Paper is a material made of cellulose

pulp derived mainly from wood or rags or certain grasses. The Chinese lost the secret of paper fabrication to the Arabs during a war in 751 A.C. Spain was the first European country to produce paper in 1151. Switzerland was mentioned in 1411, and it seems that in Germany the first paper factories existed at the end of the 14th century. Later England in 1494 and America in 1600 joined the list of paper producers. After the invention of printing with mobile letters by Gutenberg in Germany and of the cylinder former in Holland, paper production grew exponentially during the 15th century. Since then the main problem has been the availability of raw materials. Thus, rag and cotton fibres have been substituted by wood pulp in 19th century. The first chemical pulping method (using the soda process) was introduced in 1857 by Houghton; it yields raw cellulose [Kübler, 1949; Payot, 1938; Grant, 1937].

Cellulose can be extracted from different raw materials (plants, animals, and even minerals), and can also be synthesized, but up until today wood pulp is by far the most important (and economic) source of fiber in paper manufacture. Many additives are added to the paper pulp during the process to increase fiber cohesion or improve the paper properties. Bleaching was originally carried out with chlorine, which has gradually been substituted by chlorine dioxide, ozone, or hydrogen peroxide for environmental reasons. A few of the functional additives to be mentioned here are sizing agents (e.g., alum/rosin size, alkyl ketene dimers, alkenyl succinic anhydride), dry-strength agents (e.g., cationic starch, gums, polyacrylamides), wet-strength resins (e.g., urea-formaldehyde, melamine-formaldehyde, and polyamine resins), colouring and tinting agents (e.g., acid, basic and direct dyes, and coloured pigments) and the fillers or coating agents (e.g., titanium dioxide, sodium carbonate) [Scott et al., 1995; Levinson, 2001; Göttsching, 2004]. Brighteners have been introduced in the 1950s. Every paper manufacturer has his secret recipe, and therefore the composition can vary greatly. For this reason paper has a complex surface with highly variable qualities, both physical (through the fibre distribution network) and chemical (through a large choice of additives).

1.2.2 Ink

Writing ink was used in Egypt for writing on papyrus. These early inks were composed of a carbonaceous compound base extracted from cephalopoda (e.g. squids) or in China carbonized organic substances [Lucas, 1945; Levinson, 2001]. Iron-gallotannate inks (nutgalls and tannin) have been widely used since the early 12th century, and when combined with iron salts are blue. Vanadium salts and aniline used in the late 19th century are no longer in common use. Today, inks are mostly water-based (fountain pens, fibre pens, inkjet printers) or glycol-based (ballpoint pens). The laser printer process works with hot deposition on paper and requires no solvent.

Ballpoint pens were developed in Europe in the 1930s by Biro and commercially produced since 1944 in the USA. Until the 1950's, the inks contained iron gallotannate or washable dyes with oil-based solvents. Since 1950, most inks are glycol-based and copper phthalocyanine pigments were introduced in 1954 [Levinson, 2001].

Ballpoint pens, now the most common instruments for writing on paper, consist of a housing, a ball, and a container. To avoid slow drying and broadening of written lines, smudging, and fading, ballpoint inks have been developed as special mixtures of glycol solvent, colouring agents (dyes and pigments), anti-corrosives, waterproofing agents, coagulants, oleophobic and other additives, which make inks a closely-guarded industrial secret. Some manufacturers make inks, some make pens, some make both, which further complicates an already dynamic and broad market. Ballpoint ink is mainly manufactured in Japan, Germany, the USA, and China. Examples of ink formulation were reported by Brunelle [Brunelle and Reed, 1984; Brunelle and Crawford, 2003]. The present work concentrates on the dye and solvent components.

1.2.3 Ink analysis

Documents are also a support for traces of other types such as fingerprints, DNA, drugs, or explosives residues. The forensic expert must define his priorities in each case encountered. The first tests are always visual. Optical methods have the advantage of being easy, fast, and non destructive. Various standardized light sources in the visible, ultraviolet, and infrared are used with a number of filters for preliminary observations. Macroscopy, microscopy, and digital photography are powerful tools for a closer examination of all the particularities of a document. Very good results can be obtained; inks can be differentiated by determining their optical properties.

Chemical tests imply a destructive analysis, which must be allowed by the court, and the procedures may last several weeks. The document examiners will then apply one or more of the following methods with the aim of answering queries of the judge, the prosecution, or the defence: HP-TLC (High-Performance Thin-Layer Chromatography), which is widely used to determine the chemical composition of dyes and pigments; lately methods such as HPLC (High-Pressure Liquid Chromatography) and mass spectrometry, which give better resolution but are more expensive and not always available in the document examiner's laboratory; Raman spectroscopy and MSP (Microspectrophotometry), which are common tools of comparison in the analysis of the absorption properties of colorants; or GC/MS (Gas Chromatography / Mass Spectrometry), which is the method of choice for the analysis of volatile compounds, but is not generally performed as a routine.

1.3 Time

In criminalistics, time is an essential criterion at all levels of the investigative process [Margot, 2000]. Crime scene investigations have to be quick, otherwise important information can be lost (problem of persistence of the traces). If

serious serial crimes are committed somewhere, it is important to find the authors before they have time for new crimes. When a suspect is arrested, identification must be available within time restraints so that he can be held in custody. The time frame that is available is a major factor, and in the investigative process, even the best information is useless if it arrives late [Ribaux, 2004; Zingg, 2004].

Forensic scientists, like archaeologists, try to reconstruct the past; to some extent, they also try to prevent future crimes. It is not an easy task to respond to the multiple questions that can arise in the investigative process: “What? How? Why? Where? Who? When?”; especially if the investigator was not there during the event or if it has not happened yet.

Routinely, document examiners are confronted with the time problem, as they are very often asked how old an ink entry or a document is. Often the question is formulated as follows: has this entry really been written at this date, or was it written at a posterior/anterior date? The time frame may be several years or a few months. Many forensic scientists have tried to find methods that could be helpful in answering this question (see Chapter 2.2). Every book about document analysis has a chapter or paragraph about dating methods [Osborn, 1910; Brewester, 1932; Locard, 1959; Grant, 1937; Harrison 1966; Ellen, 1997; Levinson, 2001]. The aging of the ink matrix on uneven paper surfaces is a very complex physical and chemical process that is influenced by many factors. In many publications, methods for the dating of inks have been reported or proposed, but none is actually validated internationally, and only a few scientists dare to use them in court [Starrs, 2000].

1.4 Mass spectrometry

Mass spectrometry is a powerful analytical technique that is used to identify unknown compounds, to quantify known compounds, and to elucidate the structure and chemical properties of molecules. Ions are formed in the ion

source, and are then separated according to their mass-to-charge ratio (m/z) in a mass analyser. The detection of the ions is usually performed with a secondary electron multiplier (SEM) or a microchannel plate (MCP). The detection limits of mass spectrometry are low (down to the attomole range), and resolution may be as high as one to several millions, depending on analyser type and method used.

Mass spectrometry had its beginnings in 1887 in Cambridge, when J. J. Thomson demonstrated the existence of electrons and positive ions in a tube under vacuum [Grayson, 2002]. Thomson received the 1906 Nobel Prize in Physics “in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases”. Around 1920 in Chicago, A. J. Dempster developed a single focussing magnetic deflection instrument and the first electron impact (EI) source, both still commonly found as instrumental methods today. During World War II, the double focussing magnetic sector instrument has been developed by A. O. C. Nier for separation (isotopic analysis) of the uranium 235 needed for building the first atomic bomb. In 1946, the concept of Time-of-Flight (TOF) mass analysers was proposed by W. E. Stephens in Pennsylvania. Direct coupling of gas chromatography (GC) and mass spectrometry was achieved in the mid-1950s; the quadrupole mass filter proved to be adequate for this purpose as introduced by W. Paul in Bonn (Nobel Prize in Physics 1989). Lately, the triple quadrupole, the Ion Trap (IT), and the Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometers were found to be ideal for tandem MS analysis; here a precursor ion is mass-selected and fragmented, typically by collision-induced dissociation (CID), to elucidate its structure.

New ionisation techniques such as Field Ionisation (FI), Secondary Ion Mass Spectrometry (SIMS), and Fast Atom Bombardment (FAB) have found their applications in chemistry and biochemistry. The recent developments of Electrospray Ionisation (ESI) by J. Fenn (Nobel Price in Chemistry 2002) and Matrix Assisted Laser Desorption Ionisation (MALDI) by F. Hillenkamp and M. Karas had a major impact on the use of mass spectrometry in studies of large

biomolecules, and recently became an essential analytical tool in biology and medicine.

In the forensic sciences, mainly GC/MS has been employed, apart from other methods such as Inductively Coupled Plasma (ICP)-MS for trace-element analysis, Isotopic Ratio (IR)-MS for explosives analysis, or Liquid Chromatography (LC)-MS in toxicology. Many forensics laboratories are busy with daily expertises for the police, the courts, or private clients. Typically, there is not much time and money for research in these laboratories, therefore, mass spectrometry usually becomes an option, only when their routine techniques fail. However, previous research has shown that the methods of mass spectrometry have a large potential for the analysis of ballpoint dyes [Sakayanagi et al., 1999].

1.5 Aims

The final aim of this work was that of determining the feasibility of the ink dating methods used as of today. For this reason, the aging processes of dyes and solvents commonly used in ink formulations were studied. Ink entries can only be dated with an understanding of the aging mechanisms and the factors influencing the aging of the ink compounds. Until now, very few systematic studies that could help to understand the fundamentals of these complex processes are available. A few papers were published in non forensic journals in the fields of colorant quality and the food industry.

Ink aging really is a very broad and complex domain, and many forensic scientists confronted routinely with the age question have tried – besides their routine work – to develop dating methods, but were unable to validate them. Time is a decisive factor in the development of such a method, since the aging processes, apart from their inherent complexity, depend on many factors such as storage conditions and ink composition. The influence of these factors is largely unknown to the forensic community, and a large amount of time would

be required to study them exhaustively. The task is made even more complex by the diversity and rapid evolution of the ink market, which strongly depends on the availability, price, and quality control of the components involved. Few forensic labs in the world possess an up-to-date collection of samples from the ink market in their country. In this respect, the author is only aware of the Landeskriminalamt in Bayern, Germany, and the US Secret Service in Washington, USA. Such a collection requires very good contacts to the industries and a large expenditure of time to keep up with the continual changes that might occur in any new batch. Even then, the database cannot possibly be complete. Moreover, the resources that would be required to determine all aging processes of all types of pen under all possible conditions simply do not exist. An additional problem in studies of the aging of ink is the availability of controlled samples of old batches.

As a starting point toward an improved understanding of the aging of inks, the present work had the aim of better defining the processes involved, as well as the chemistry behind them. For this reason, several blue ballpoint pens were selected randomly on the German market. Ballpoint pen entries were then aged over 2 to 3 years under defined storage conditions so as to obtain an aged sample batch. In a first phase, mass-spectrometric methods (LDI-MS, MALDI-MS, GC/MS and ESI-MS) and protocols have been developed, tested, evaluated, and validated for the analytical characterization of ballpoint ink on documents. Spectroscopy was also used for dye analysis. In a second phase, these methods could then be used to study precisely those aging processes that are characteristic for the ink, such as the fading of triarylmethyl dye and the drying of glycol solvents on paper substrates. All the factors influencing these aging processes were identified, and for most of them, the extent of their influence was determined experimentally. Aging pathways and products were identified. Measured quantities that depend on age were then defined so as to determine the kinetics of the reactions and produce aging curves for both dyes and solvents. The potential use of ballpoint ink dyes and solvents in the dating of questioned documents was tested, and the applications and limitations were determined. In addition, a small part of this work was devoted to the

development and testing of Scanning Microprobe LDI-MS for determining the crossing sequence of lines, which can also be helpful in finding the relative age of an ink entry.

The results of this work are intended to give forensic scientists a better understanding of the complexity of the aging processes involved in a ballpoint ink matrix on a porous substrate such as paper. Such a fundamental study of the aging processes is new in forensic science, and was needed to comprehend how a dating method should be developed and validated in order to provide useful and genuine results. A strategy to set up dating methods is also proposed at the end of this document.

Ich wundere mich immer über mein Beginnen. Über das, was aus meinem Kopf in den Kugelschreiber fliesst.

I always wonder about my commencement. About what flows from my head to my ballpoint pen.

J.R. Von Salis

2 THEORY

2.1 Composition of ballpoint ink

Ballpoint ink contains the following major compounds [Weyermann 2003b; Bügler 2005]: solvents (50%), dyes and pigments (25%) and resins (25%). Other ingredients are present in small quantities and include lubricants, biocides, surfactants, corrosion-inhibitors, sequestrants, shear-thinning agents, emulsifying agents, buffers and many other minor additives to adjust pH, viscosity, polymerization and prevent pen blockage or microbial growth in the ink [Brunelle and Crawford, 2003]. For example, aryl guanidines (Fig. 2.1) are bases used to form salts with acid dyes and raised the pH of ink [Ng et al., 2002].

2.1.1 Dyes and pigments

Natural dyes can be of inorganic (mineral) or organic (biologic) origin, the former having the advantage to be absolutely photostable to the extent that their colour results from atomic transitions in stable crystals. Unfortunately their potential applications are limited by their availability [Suppan, 1994]. For these reasons, organic (biologic and synthetic) dyes have been used to lend colour to normally colourless materials (e.g. ink or cloth). Basic dyes based on triphenylmethane were amongst the earliest synthetic dyes to be discovered [Allen et al., 1980; Hunger, 2003]. Dyes are coloured, ionic aromatic organic compounds. As such they are based on the structure of the benzene molecule that absorbs electromagnetic radiation in the ultraviolet wavelength range (at about 200 nm). Visible light ranges between 400 to 800 nm in the electromagnetic spectrum. The visible and ultraviolet spectra of organic compounds are associated with transitions between electronic energy levels in

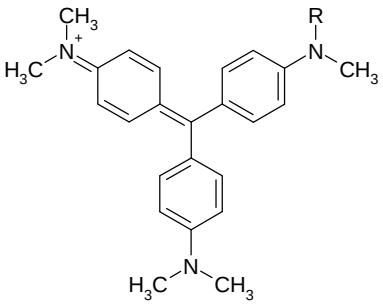
the molecules. The colour of dyes is a consequence of the presence of a chromophore (π electrons acceptor) altering the energy levels in the delocalised electron cloud of the dye molecules. This alteration results in the compounds absorbing radiation within the visible range of the electromagnetic spectrum, and our eyes detect that absorption as a colour. Moreover, auxochromes (meaning: *colour increaser*; π-electrons donors) are able to become attached to non ionising compounds while retaining their ability to become converted into ions, thus affecting the absorbance of the resulting compounds. Colour is often due to a charge-transfer-type electronic transition of relatively low energy corresponding to an absorption in the visible region of the spectrum [Hesse et al., 1991; Williams and Fleming, 1997].

The dyes and pigments are the colorant components of ink contributing their colouring properties. Dyes usually are used as an aqueous solution, and may require a mordant to improve the fastness (i.e. stability) on a substrate. In contrast, pigments are insoluble and generally have no affinity for the substrate. Many colorants exist on the market; those for ballpoint ink must have a strong, lasting colour and low price. The dyes are mainly cationic (or basic) dyes. The charge-carrying atom usually is nitrogen and the charge may be localized or delocalized. Figure 2.1 shows some typical blue dyes found in ballpoint pens [Sakayanagi et al., 1999; Ng et al., 2002]: Basic Violet 3 (hexamethylated methyl violet), Basic Violet 1 (pentamethylated methyl violet), Solvent Blue 2 (Neptun Blue), Basic Blue 26, Basic Blue 7, Basic Violet 10 (Rhodamine B) and copper phthalocyanine (Pigment Blue 15) derivatives. Anionic (or acid) dyes are also in ballpoint pens ink, but less commonly.

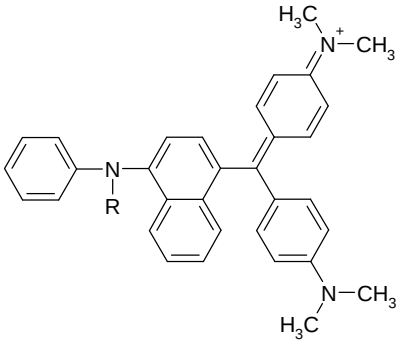
2.1.2 Solvents

Solvents are contained in ink for two main reasons: dilution of the colorant and its application on paper. Glycol solvents are the solvents most commonly used in ballpoint inks, as such an ink must be more viscous than water to fulfil the requirements of a ballpoint pen. They allow the ink to stay fluid in the pen

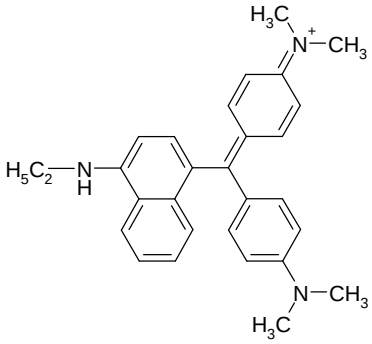
cartridge but dry quickly on paper after application. Lubricants such as oleic acid are added to permit the ball to rotate freely. Several examples of typical solvents found in ballpoint pens are shown in Figure 2.2 [Fortini, 2000; Brunelle and Crawford, 2003]: phenoxyethanol, phenoxyethoxyethanol, dipropylene glycol, benzylalcohol, butylene glycol, phthalic anhydride, oleic acid and 2-pyrrolidone.



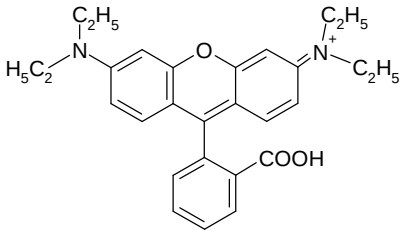
R=CH_3 , Basic Violet 3 (CI 42555, 372 g/mol)
R=H , Basic Violet 1 (CI 42535, 358 g/mol)



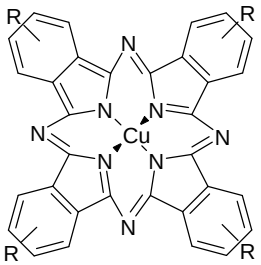
R=CH_3 , Basic Blue 2 (CI 50240, 484 g/mol)
R=H , Basic Blue 26 (CI 44045, 470 g/mol)



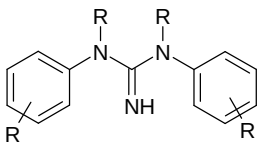
Basic Blue 7 (CI 42595, 478 g/mol)



Basic Violet 10 (CI 45170, 443 g/mol)



Copper Phthalocyanine (CI 74160, 575 g/mol)
and derivatives (e.g. R=HSO_3)



R=CH_3 or H, Aryl Guanidines
(268, 240, 226, 212 g/mol)

Figure 2.1 Structure of cationic dyes (with their Colour Index, CI) typically used in ballpoint inks: basic violet 3, basic violet 1, basic blue 2, basic blue 26, basic blue 7, basic violet 10, copper phthalocyanines derivatives and aryl guanidines.

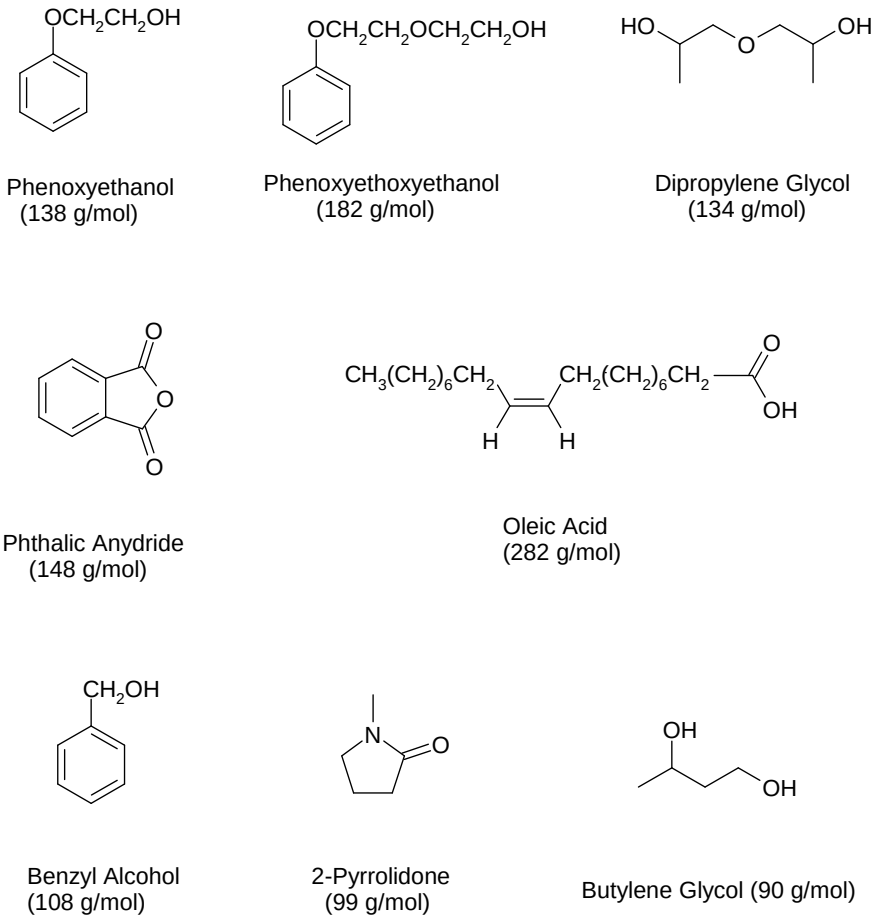


Figure 2.2 Structure of solvents typically used in ballpoint inks: phenoxyethanol, phenoxyethoxyethanol, dipropylene glycol, phthalic anhydride, oleic acid, benzyl alcohol, 2-pyrrolidinone and butylene glycol.

2.1.3 Resins

Resins are substances of relatively high molecular weight synthesized by polymerization of monomers. They are added to the ballpoint ink formulation for adjusting the viscosity of the ink and increasing film strength and lubricant qualities as the ink flows from the ball onto the paper [Brunelle and Crawford, 2003]. Resins additionally create a bond between the ink and papers while they polymerize during the drying process. They are insoluble in water, but soluble in

the organic solvents contained in the ballpoint ink formulation. Some examples of resins used are: ketone, sulfoamide, maleic, xylene, alkyd, phenolic and rosin resins, ester gums, styrene and allyl alcohol [Brunelle and Crawford, 2003], oleylamine etoxylate, phthalic acid ester, hydrogenated acetophenone, formaldehyde condensate [Kirsch et al., 2005a].

2.2 Aging processes of ballpoint ink

It is usually admitted that in a cartridge, the ink undergoes very slow changes or no change at all [Grim et al., 2002]. Once ink is applied on paper, the aging processes start: the solvents migrate into the paper and evaporate, the dyes fade, and the resins polymerise. Some factors such as light exposure, humidity and temperature may influence the aging, and it cannot be ruled out that paper composition, too, may play a role. The aging processes are also influenced, both physically and chemically, by the paper's complex composition (see Chapter 1.2.1 above) and by cellulose decay [Bansa, 2002] as will be discussed below.

2.2.1 Dyes

When dyes fade, they undergo a photochemical reaction that begins with the absorption of visible or ultraviolet light. Absorption of a photon by an organic molecule leads to formation of an electronically excited state that is the starting point for subsequent reaction steps [Coyle, 1986; Becker et al., 1991]. Photochemical reactions are influenced by the concentration of the reactants, the reaction medium, the temperature, the wavelength and intensity of the light [Klessinger and Michl, 1989].

Triarylmethane dyes (e.g. methyl violet) are favoured as colour formers in ink because of their low cost and strong colour, but they are characterised by

relatively low photostability and their intensity fades with time and exposure to light [Caine et al., 2002; Brezová et al., 2003]. Fading is influenced by the structure of the dye as well as by external and environmental parameters. Thus oxygen, moisture, temperature, agents such as air contaminants (sulphur dioxide and nitrogen oxides from pollution), the wavelength of incident light, and concentration of the dyes influence the rate and pathways of photofading [Egerton and Morgan, 1970; Keuch, 2003; Keuch, 2004]. The surface properties, the chemical and physical structure of the substrate, residual solvent within the substrate, and porosity also may quite significantly influence the photofading [Brezová et al., 2003].

In textile industry, it is well known that triarylmethane dyes have poor light fastness in cotton and wool, while to the contrary they yield very strong and stable shades in acrylic fibre [Allen et al., 1980].

Photochemical processes of degradation of the dyes may follow different pathways, and a wide range of different products may result. This topic has mainly been studied for industrial purposes, in order to find ways of quenching the fading of cloth, inks, or paper dyes without increasing the costs. Conservation scientists have also been studying easel and fresco paintings for decades, because the mixtures of dyes, pigments and diverse molecules used undergo complex transformations while aging [Wyplosz, 2003]. Dye neutralisation has been widely studied in environmental sciences, since it is important that toxic dyes be photodegraded [Li et al., 1999] or biodegraded [Sarnaik and Kanekar, 1999] before they are released into the environment. The deactivation pathways of excited states of the dyes represent interactions with their environment, since many of the radicals involved are produced from the solvents or the substrates upon exposure to light [Brezovà et al., 2003]. Thus, the photodegradation of the triphenylmethane dyes is accelerated by the presence of singlet oxygen sensitizers (e.g. methylene blue or titanium dioxide), but retarded by singlet oxygen quenchers (e.g. β-carotene or zinc(II) and nickel(II) complexes), demonstrating the involvement of singlet oxygen in the degradation processes [Caine et al., 2001].

Several different fading reactions may occur [Egerton and Morgan, 1970; Kuramoto and Kitao, 1982; Li et al., 1999; Sarnaik and Kanekar, 1999; Caine et al., 2001; Grim et al., 2000; Weyermann et al., 2002; Brezová et al., 2003]:

a) *N*-Demethylation (Figure 2.3) has been studied by many authors, as it is very easily detected. The methyl groups of the dye are sequentially replaced by hydrogens (mass difference of 14) upon exposure to light.

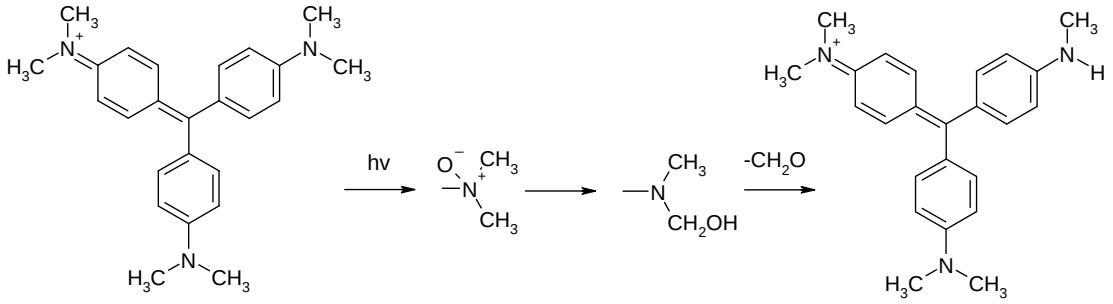


Figure 2.3 Mechanism proposed by Caine et al. [2001] for the *N*-demethylation of methyl violet.

b) Photooxidative cleavage of the central C-phenyl bond, probably via singlet oxygen, to give benzophenones and phenols (Figure 2.4). It has been demonstrated that triarylmethane dyes produce singlet oxygen upon photolysis on paper, but not necessarily in ethanol or water [Brezovà et al., 2003]. Ring opening by OH radicals formed by singlet oxygen in water has also been proposed [Li et al., 1999].

c) Photoreduction of an excited state dye cation to a colourless leuco-dye form by addition of an electron to the photoexcited species or by photochemical hydrogenation of the dye.

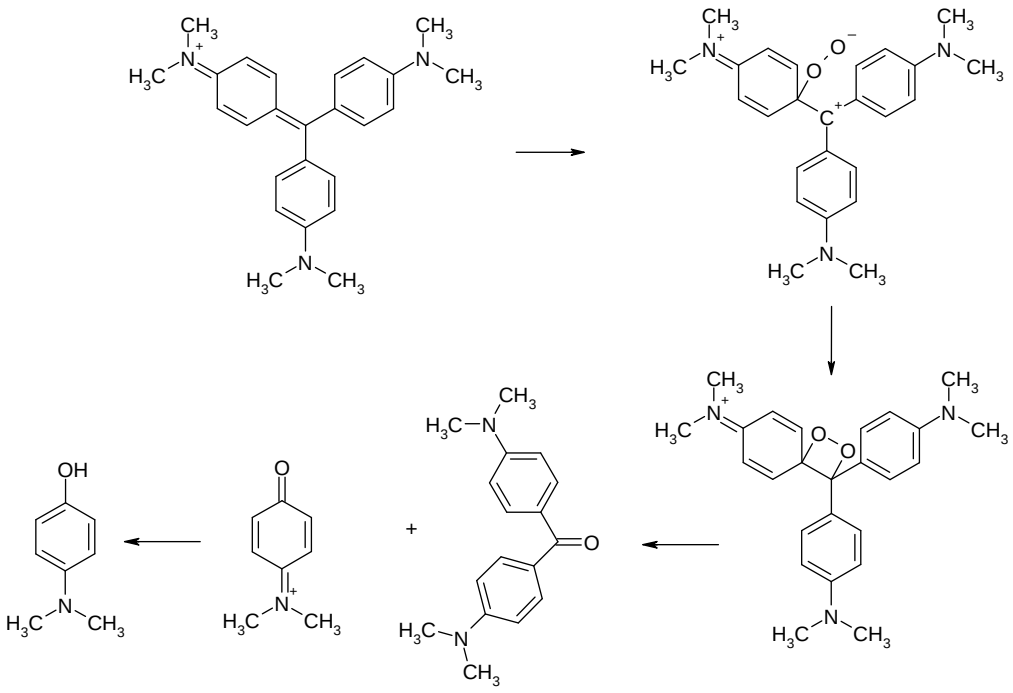


Figure 2.4 Mechanism proposed by Henriquez in 1933 [Egerton and Morgan, 1970] and redefined by Kuramoto and Kitao [1999] for the degradation of methyl violet through singlet oxygen attack, which produces dimethylaminobenzophenones and dimethylaminophenol.

All these degradation reactions may occur under the same experimental conditions, and compete with each other. Contradictory results have been reported concerning the degradation of ballpoint dyes in the absence of light [Aginsky, 1995; Grim et al., 2001; Andrasko, 2001; Ng et al., 2002]. These deviations could eventually be explained by different storage conditions (room temperature, humidity, substrate), different time periods over which the studies were carried out, and different analysis methods.

Ballpoint inks usually are complex mixtures with different additives, solvents, resins, and papers can also be very different in their structure and chemical composition. Moreover storage conditions such as the amount of light, the temperature, humidity and air composition influence the fading pathways and kinetics. It is essential, therefore, to thoroughly study the aging processes of triarylmethane dyes while accounting for the many factors of influence in order to understand the fading of dyes on the paper matrix.

2.2.2 Solvents

Drying is a very complex phenomenon characterised by evaporation of the solvents in the ambient air and their simultaneous adsorption on and diffusion into the paper. Among other things, these processes are influenced by temperature, humidity and the adsorption/diffusion properties of the paper-solvent system. In earlier forensic studies [Stewart, 1985; Aginsky, 1996], the simplifying assumption had been made that the following elements:

- storage conditions (temperature, humidity, adjacent material)
- paper properties (pore size, coating, pH)
- composition of ink (solvents, dyes, resins, and the set of additives)

have no decisive influence on the aging curves (or drying rate), and that threshold values of ink aging parameters can still be used to decide whether an ink entry is fresh or old, without knowledge of these factors. When considering the basic principles of the theory of drying, one can easily see that these factors actually cannot be neglected, and that doing so will lead to discrepancies in the interpretation of the results. The importance of assessing many additional variables when evaluating the drying process has recently been mentioned by White [White, 2004].

In principle, drying processes constitute a simultaneous mass and heat transfer, while the thermal energy needed to evaporate a liquid from a porous solid is provided by the ambient air [Avcı et al., 2001]. For the purposes of a closer analysis, the drying process can be separated in three phases [Avcı et al., 2001; Bird et al., 1960; Ondrastschek et al., 2001; Strobel, 2004]:

- *Increasing rate of drying:*

  In this phase, the evaporation rate increases as the wet external surface area grows through lateral diffusion along the paper fibres. This process can be neglected for very small quantities.

- *Constant rate of drying:*

  This phase begins when the evaporation rate and the surface area reach a stationary state, and equilibrium conditions occur at the free surface.

- *Falling rate of drying:*

  In this phase, the migration of solvents from the bulk of paper towards the surface becomes slower than the evaporation rate at the surface (which is now unsaturated). Two mechanisms operate here: The evaporation surface recedes into pores (1^{st} falling); later, capillary migration stops (by increased physical adsorption in cellulose fibres), and evaporation occurs within the paper (2^{nd} falling).

Due to the fact that diffusion and adsorption (physisorption) mechanisms play such an important role in the drying of solvents on porous media, a wealth of external factors must be taken into account. Among these are temperature (of air, solid, ink), vapour pressure (air, solvents), air movement (laboratory, cabinets), the properties of solvents mixtures (vaporization of the solvent mixture, viscosity), paper and ink properties affecting heat transfer and mass transfer coefficients. The drying time in particular would reflect this situation, and in turn would depend on these parameters. Theoretical drying rate equations have been proposed, but since most of the factors involved are difficult to define theoretically, these equations are of limited applicability, and additional empirical measurements are needed to follow the drying of ink on paper.

Lociciro et al. [2004] reported a loss of 89 to 98% of phenoxyethanol from the ink entries within a few minutes, and attributed this loss to evaporation. In other work, Selim et al. [1997] reported a rate of penetration of the solvents into the

paper that was at least 20 times higher than the rate of evaporation found for water-based inks. This is consistent with the objective of ballpoint pen manufacturers, of producing a fluid ink that is easily applied on paper (low friction between ballpoint and paper) while at the same time drying very quickly at ambient temperature (to avoid smearing of the ink after deposition). In view of these requirements, solvents (contrary to dyes) are not meant to remain in the ink entries for years, but should only aid the application of the ink on paper. In fact, the solvents quantities deposited typically are in the microgram range [Weyermann et al., 2003 a; Weyermann et al., 2003 b], and decrease very quickly.

2.3 Dating of ballpoint ink

Many reviews on dating methods have been published over the last 30 years [Brunelle and Cantu, 1987; Stewart and Guertin, 1991; Brunelle, 1992; Brunelle and Lee, 1992; Lothar, 1992; Hicks, 1993; Aginsky, 1993; Stewart et al., 1995; Dormann, 2000; Brunelle and Crawford, 2003; Jahns, 2004], thus, only a summary will be given here. Two fundamental approaches can be distinguished: the static approach [Cantu, 1995] focussing on production dates, and the dynamic approach [Cantu, 1996] focussing on aging processes of inks. Age determination of documents has been a subject of thorough studies, at least since the beginning of this century. When ink compositions change, some methods can no longer be used, and new ways must be investigated.

2.3.1 Static Dating

The static approach is based on the compositions of ink and paper on the market that have changed along the years (see Section 1.2). Ballpoint inks appeared on the market in 1945, and had oil-based solvents until 1951 when glycol solvents were introduced. Erasable ballpoint ink was invented in 1963,

pressurized ink in 1968. In 1954, copper phthalocyanine dye was included in the composition. Between 1969 and 1991, the Bureau of Alcohol, Tobacco and Firearm initiated an ink tagging program in the USA, by inserting fluorescent tags to the ink which made it possible to determine the year in which a particular ink was brought onto the market. This process is time-consuming and expensive, and requires collaboration of the ink industry. The US Secret Service resumed such a program in 2002 [LaPorte, 2004].

Databases for ink compositions were established by the US Secret Service and by the Landeskriminalamt of Bayern (Germany) in collaboration with ink manufacturers. It is a very demanding task, and no database can ever be entirely up to date. Since dyes fade and solvents disappear, the composition of an ink entry is not constant over time. Thus, only inalterable characteristics should be taken into account, such as pigments or inorganic trace elements composition [Montero, 2004].

Very often, paper plays an important role in the static age determination, as this is difficult to imitate. The best-know case is that of “Hilters Tagebücher” (Hitler’s diaries) that reappeared in Germany near the end of the last century. By handwriting comparison, the conclusion had been reached that Hitler was indeed the author of these notes. A statement contested by Julius Grant, who reported that fluorescent optical brighteners were found in the paper of the diaries pages [Grant, 1985]. To expose these diaries to an ultraviolet light source certainly was one of the easiest expertises of his life. In fact, in West Germany, these agents were actually added to paper composition only in 1948, well after Hitler’s death [Göttsching, 2004]. The handwriting comparison failed to reveal the fraud, because the writing was indeed the same: the comparison documents were written by the forger of the diaries himself.

Today, watermarks and micro-impressions in quality papers may include a date of fabrication, thus providing the earliest possible date of entry. A determination of isotope composition, such as ^{14}C analysis, is not very useful, as it could only provide an approximate date (within a range of about ten years) for the felling of the tree (and then assuming that the papermaking process is known) [Mildenhall, 2004].

2.3.1 Dynamic Dating

These methods rely on quantitative measurements of physical (e.g. motions) or chemical (e.g. reactions) changes of the ink as a function of time, i.e. producing an aging curve for that particular ink. The aging processes measured must be reproducible under given conditions in order to insure a correct determination of the date of entry. Thus, the first step is that of determining aging curves (measuring the changes as a function of time) while taking into account the factors influencing the aging. The measuring errors should not be larger than predictable variations, and blind tests should confirm the reliability of the method.

The introduction of new ink composition along the years also meant changes in the aging processes. Some of the older methods such as chloride or sulphate migration from the ink into the paper [Türkel, 1933; Metzger et al., 1933; Heess, 1935; Heess, 1937] can no longer be used, as most inks are now free of these ions. Earlier gallotanic inks also were acidic, and caused paper deteriorating. Moreover, they contained iron that oxidised, provoking a change of colour [Brewster, 1932; Osborne, 1910; Lucas, 1945].

Absolute versus relative dating

The main problem arising in the attempt of determining the absolute age of an ink is the dependence of the aging processes on the storage conditions and initial composition. Effectively, these variables are rarely available in document expertise. For this reason, relative dating usually is the only reliable way to date documents. If two ink entries from the same pen have been stored under the same conditions (e.g. diary or notebook), it will then be possible to comparatively determine which one is older. Considering that in all other cases a dating is difficult or even impossible, Cantu [1988] proposed a method of

relative dating which only requires a single ink entry. The method is based on artificial aging, a process in which the ink stroke is exposed to conditions (such as light, heat, water) accelerating the normal aging process. It is assumed that these procedures can lead to an aging curve when an ink entry is analysed before and after being artificially aged. The utilisation of this approach has been widely studied and discussed for the purposes of forensic documents examination [Osborne, 1910; Stewart, 1982; Lyter, 1994; Aginsky, 1996; Stewart and Fortunato; 1996; Brunelle and Speck, 1998; Grim et al., 2002]. In the USA, Aginsky [1996; 1998] developed this method further, by including measurements of artificial solvent drying rates for the routine dating of ink entries.

Extractability of ink from hardened resins

Changes in the extractability of the ink caused by the hardening of the resins have been investigated by measuring the dissolution rates in acids. This method was proposed by Kikuchi in 1959 [Locard, 1959; Hicks, 1993]. Many authors have measured the sequential dissolution or extraction of dyes into weak and strong solvents by Thin-Layer Chromatography (TLC) [Kuranz, 1986; Brunelle et al., 1987; Brunelle and Lee, 1987; Cantu and Prough, 1987; Aginsky, 1994; Brunelle, 1995]. Some authors later reported that these measurements were not reproducible for reasons such as the fact that every ink would require a different extraction solvent, and give a different aging curve [Aginsky, 1998; Hicks, 1993, Hicks Champod et al., 1995; Andermann and Neri, 1998; Jahns, 2004]. Aginsky developed a similar method based on sequential extraction of solvents from ink entries by GC/MS [Aginsky, 1998].

Fading of dyes

As the fading of dyes through light exposure is visible to the naked eye, many methods have been developed to measure the degradation with time: Thin-Layer Chromatography (TLC) [Sen and Gosh, 1971; Aginsky, 1994],

MicroSpectroPhotometry (MSP) [Aginsky, 1995], Capillary Electrophoresis (CE) [Fanali and Schudel, 1991; Vogt et al., 1999], Particle Induced X-Ray Emission (PIXE) [Vogt et al., 1999], High Performance Liquid Chromatography (HPLC) [Andrasko, 2001a, b; Kher et al., 2001; Mitchell et al., 2002; Bügler, 2005; Hofer, 2004b], Fourier Transform Infrared (FTIR) Spectroscopy [Wang et al., 2001], Raman Spectroscopy [Claybourn and Ansell, 2000], and Mass Spectrometry. Mass Spectrometry (MS) has been evaluated early for the forensic examination of fiber-dyes and varnish aging [Bennett and Schweikert, 1989; Eichhoff and Opitz, 1973].

It can be seen that many modern methods exist, but they are not always available to the forensic scientists. Some of them may be more sensitive, precise, and reproducible than others, but all of them yield a qualitative determination of the dyes found in the ink. When ink degrades, new colored and colorless substances are produced quantitatively as functions of time, and can be detected by mass spectrometry. In recent years, many MS methods have been investigated for the purpose of identifying dyes and dating of inks: Field Desorption (FD) [Sakayanagi et al., 1999], Secondary Ion Mass Spectrometry (SIMS) [Lyter, 1999], Laser Desorption Ionisation (LDI) [Grim et al., 2000, 2001; Weyermann et al., 2002, 2003b; Wyplosz, 2003], Electrospray Ionisation (ESI) [Ng et al., 2002].

Unfortunately for the forensic scientists, dyes which are unstable in the presence of light do not degrade in the dark, or only very slowly so. Therefore, dating relying on dye degradation usually is carried out, only by comparing ink entries from the same pen that were stored under the same conditions (e.g. diaries and notebook). Researchers still carry on with the hope of optimizing such methods.

Crossing sequence of lines

In many cases, microscopy has failed in determining the crossing sequence of lines, and other methods had to be developed. It is quite common that analytical problems arise when the ink entries are physically and chemically mixed where

strokes cross. For this particular reason, no method can lead to results that are satisfactory and reproducible in every circumstance. Fourier Transform Infrared (FTIR) and Raman Spectroscopy [Becker and Brunelle, 1984; Wang et al., 2001; Mania et al., 2003], Scanning Electron and Atomic Force Microscopy [Stitt et al., 2003], Laser Profilometry [Berx and De Kinder, 2003], Scanning Microprobe LDI [Weyermann et al., 2004 b] are confronted with the same issues.

Drying of solvents

Lately, interest was revived into a method first proposed by Stewart in 1985 [Stewart, 1985], in which evaporation of the volatile components of ink is measured using GC/MS. The Forensic Division of the Canada Customs and Revue Agency (CCRA) [Brazeau and Gaudreau, 2003], the Swedish National Laboratory of Forensic Science (SKL) [Andrasko, 2003a], the Bavarian State Bureau of Criminal Investigations of Münich (LKA Bayern) in Germany [Bügler, 2005], the University of Münster in Germany [Jahns, 2004] and the State Police of Zürich in Switzerland [Hofer, 2004a] have reported encouraging research peformed by them in this field. Interestingly, however, three different studies on one hand have indicated that a dating of ink by this method becomes impossible after a few days [Fortini, 2000; Lociciro et al., 2004; Andrasko, 2003 b], while on the other hand Aginsky, and recently a research group based in China, reported very positive results for analysis conducted over longer periods of time [Aginsky, 1994; Aginsky, 1996; Aginsky, 1998, Wang et al., 2005]. These contradictory observations could possibly be explained by different methods of sample preparation and of evaluation of the results, but further studies are necessary to fully understand this situation.

Ink solvent sample preparation for GC analysis can take different forms: liquid extraction with different solvents (acetonitrile [Aginsky, 1996], dichloromethane [Fortini, 2000] or methanol [Andrasko, 2003]), derivatisation [Lociciro et al., 2004], solid phase microextraction (SPME) [Andrasko, 2003], and thermodesorption with cryo focusing [Bügler, 2004b; Hofer 2004].

In general, solvents represent more than 50% of the weight of ballpoint inks [Bügler, 2004b; Weyermann, 2003 b], and disappear from the stroke with time. The basic approach to a kinetic analysis of the solvent disappearance is the complete extraction of the solvents from the stroke, and their quantitative analysis by GC/MS at different times after application of the ink to the paper. In this way, it is usually possible to determine aging curves showing the disappearance of solvent from the ink entry with time. It is expected that this dependence of the relative peak area (RPA) over time will be affected by a number of factors other than volatility. In particular, it may depend on the total solvent mass deposited on the paper within the ink entry, and hence the extracted mass would scale with the width and thickness of the ink entry. As a way to resolve this problem, Aginsky proposed to normalise the peak area of the solvent that evaporate with respect to that of a non-volatile, stable component of the ink entry [Aginsky, 1996]:

$$\text{RPA} = \frac{\text{Area of peak of volatile solvent}}{\text{Area of stable peak}} \qquad \text{Eq. (2.1)}$$

This ratio is independent of the quantity extracted, and should in principle decrease exponentially with time. A major difficulty in this procedure is that of finding a stable non-volatile substance in the chromatogram. Aginsky proposed phthalic anhydride [Aginsky, 1996], but Fortini [2000] observed that phthalic anhydride does disappear from the stroke with time. Lociciro et al. [2004] managed to identify a stable compound in the ink by derivatising the extract with MSTFA, but this additional step probably reduces sensitivity. Also, SPME as an extraction method was found to be quick, but not quantitatively reproducible [Andrasko, 2003]. Presently, however, cryo-focus thermo desorption appears to be the method of choice, because preparative steps that may have a modifying effect are avoided. In addition, monomers readily identifiable in the chromatogram [Bügler, 2004] and yielding stable peaks are effectively extracted by this method. In the method reported most recently by Aginsky [1998], the

drying rates of solvent are measured using sequential extraction and artificial thermal aging. At normal storage temperatures, the extraction ratios found before and after artificial aging provide an indication of the drying rate, which in turn suggest an approximate age for the entry. No further details about the method were published since 1998, but it was widely used in real cases in the USA. A similar method was developed for documents expertise in Germany [Bügler, 2005].

2.4 Mass spectrometry and spectroscopy

The methods used in the present work will be briefly reviewed in order to convey a better understanding of the results presented. The mass spectrometer instruments were equipped with Microchannel Plates (MCP) for detection of the ions. A channel plate is a regular hexagonal, close-packed array of channels in a flat plate of semi-conducting material. Detection occurs through the generation of secondary electrons by collision of the primary ions with the wall. To achieve higher gain, two plates can be operated in tandem.

2.4.1 LDI-TOF-MS

Laser Desorption Ionisation (LDI) [Posthumus et al., 1978] consists of two simultaneous, linked processes. On one hand, atoms and molecules are desorbed by the laser energy from the solid sample, escaping by sublimation into the gas phase. On the other hand, ionization of the molecules occurs. The sample must absorb the wavelength of the laser light so that the molecules can be detached from the surface through a kind of thermal shock.

Matrix Assisted Laser Desorption Ionisation (MALDI) differs from LDI by the use of a matrix added to the analyte prior to analysis [Karas et al., 1987; Karas et al., 1988]. The matrix is a compound absorbing light at a given laser wavelength, which will allow compounds not themselves absorbing the laser

light to become desorbed and ionised without much fragmentation. In most cases matrix addition will improve the sensitivity of the technique [Lottspeich, 1999].

Scanning Microprobe LDI-MS [Spengler and Hubert, 2002; Bouschen 2004] is an imaging method for laterally resolved surface analysis. Through laser shots at given intervals, a surface is scanned while automatic recording and processing of the mass spectra provides images of the distribution of chemical compounds. With this method one can microscopically visualize concentration profiles of different compounds on a surface.

Time-of-Flight (TOF) is an adequate mass analyzer for the discontinuous pulsed LDI source. The ions generated are accelerated into a linear tube by an electric field. The kinetic energy E_{kin} is independent of the mass m, and is due to the acceleration potential U and the charge z of the ions (e: elementary charge):

$$E_{kin} = U \cdot z \cdot e \qquad \text{Eq. (2.2)}$$

Neglecting second order effects, the speed V of the ions entering the field free tube (drift tube) is as follows:

$$V = \frac{l}{t} = \sqrt{\frac{2Uze}{m}} \qquad \text{Eq. (2.3)}$$

where l are the added effective distances the ions have to fly in the ionisation source (acceleration distance) and the drift tube (drift distance), while t is the required time to reach the detector.

$$t = c \cdot \sqrt{\frac{m}{z}} \qquad \text{Eq. (2.4)}$$

The time of flight in a first order approximation is proportional to the square root of mass divided by the charge of the ions. The constant c is calibrated for a given instrument [Cotter, 1994; Boesl, 2003].

2.4.2 GC-MS

Capillary Gas Chromatography coupled to a Mass Spectrometer [Gohlke, 1959] offers the advantage of characterizing small amounts of complex mixtures through separation along the GC column and consecutive analysis in the MS. The basis of the GC separation is the distribution of the sample between two phases contained within the column. One of these phases is a stationary liquid, possibly coated on a bed of particles with large surface area. The other is a gas that percolates through the column and carries sample molecules. The sample must be volatile and thermally stable. Because of their different adsorption, diffusion and thermal properties, the components of the sample are partitioned between the carrier gas and the nonvolatile phase and elute differently on a particular column.

The preferred ionization method for GC/MS is Electron Impact (EI), where an electron beam produced by a filament is accelerated and collides perpendicularly with the sample, thus provoking ionization through interaction with the molecules. The fragmentation pattern of a given molecule is unique and reproducible, allowing its identification through library search.

Most GCs are coupled with a quadrupole mass analyzer consisting of four electrically conducting, parallel rods. Opposite pairs of electrodes are electrically connected to generate an electric field. Ions separation occurs by deflection caused by oscillating potentials (direct current, dc + radio frequency, rf voltages). Ions from the source are injected into the quadrupole array, where a range of kinetic energies can be tolerated. By controlling the dc / rf voltage ratio, the field can be adjusted so that only ions of a particular m/z ratio will be able to traverse the entire length of the quadrupole array to the detector. The path of all other ions oscillates with increasing amplitudes until these ions finally collide with the electrodes and are neutralised. The flight paths and forces are described by Mathieu's equations (see figure 2.5) [Budzikiewicz, 1998].

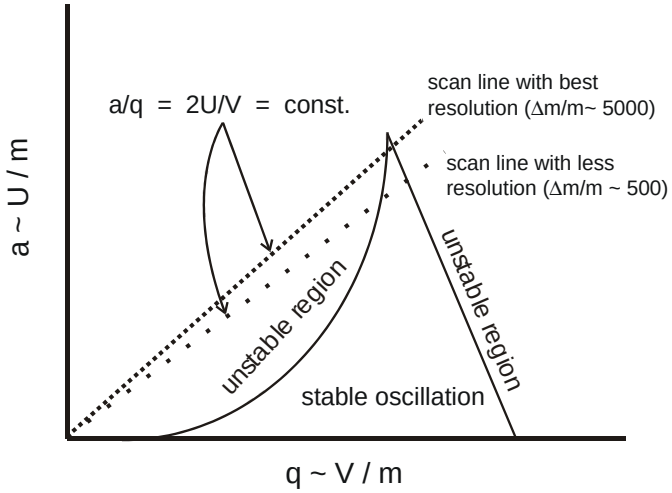


Figure 2.5 Stability diagram for a two dimensional quadrupole mass filter derived from Mathieu's differential equations. U and V are the amplitudes of the dc and rf voltage, respectively.

2.4.3 ESI-MS

Electrospray Ionisation (ESI) [Fenn et al., 1989] describes the dispersion of a solution in many tiny drops with the help of an electrostatic field. A sample solution is sprayed through a capillary glass needle. A layer of metal covers the needle and is polarized so that it has a potential against a lens, and an electrostatic field is formed between needle and lens. Small drops form at the tip of the needle, and through evaporation of the solvent, the charge density increases, provoking many Coulomb explosions until the drops collapse into single, highly charged ions. The ions then enter the evacuated mass spectrometer through a pumped inlet [Lottspeich, 1999].

In the Quadrupole Ion Trap (IT), ions can be confined using a combination of static and alternating electric fields. It consists of a set of ring electrodes that generate a quadrupolar field (rf and ac voltages), and end caps with a polarity opposite to that of the ions (dc voltages). By application of appropriate rf and dc voltages, the final position of the ions can be maintained within the center section of the ion trap. By adjusting the rf voltage, one can eliminate all but the desired ions in preparation for subsequent fragmentation via an increase of a

supplemental resonance excitation voltage while helium is introduced as collision gas. The contents of the trap are scanned for the purpose of producing mass spectra, by linearly increasing the rf voltage and utilizing a supplemental resonance ejection voltage. With the linear trap one can analyse daughter ions (fragments), and several consecutive fragmentation experiments (MS/MS or MS^n), which yield information for an identification of the parent ion, are possible [Siuzdak, 1996].

2.4.4 Spectroscopy

Spectroscopy is an adequate method for the analysis of dyes and pigments, as they absorb incident light. Two empirical laws concerning the absorption intensity have been formulated. Lambert's law states that the fraction of the incident light absorbed is independent of the intensity of the source. Beer's law states that the absorption is proportional to the number of absorbing molecules [Williams and Fleming, 1997]. The following equation results from these laws:

$$\log_{10} \frac{I_o}{I} = \varepsilon \cdot d \cdot c \qquad \text{Eq. (2.5)}$$

where I_0 and I are the intensities of the incident and transmitted light ,respectively, d is the path length of the absorbing solution [cm], and c is the concentration [mol/l]. The term $\log_{10} I_0/I$ is called the absorbance A, while ε is known as the molar extinction coefficient [L/(mol·cm)]. An absorbance spectrum can be recorded by two identical beams of light passing through a cell containing the sample and through a reference cell. The intensities of the transmitted beams are then compared over the entire range of wavelength of the instrument. If measurements are made in reflected light ($I_R = I$) mode, then the following conversion yields the absorbance:

$$A = -\log_{10}(I_R) \qquad \text{Eq. (2.6)}$$

2.5 Interpretation

The interpretation of forensic evidence is based on the *principle of transfer* or divisible matter first enounced by Locard [Locard, 1920], and the *principle of uniqueness* or individuality [Kirk, 1963]. Criminal activities, which often are intense, lead to contacts between objects and/or persons provoking the transfer of matter. A scientist using a given method will be able to conclude that two samples are identical (no discordance is observed between two samples). Saying that the two samples effectively have the same source is more difficult, as several objects/persons may produce the same traces (evidence). Many forensic studies focus on the inference of sources, by classifying potential common sources for an evidence item and attempting the individualization of a singular source for two items [Inman and Rudin, 2001]. Fingerprints or DNA have high statistical value for the individualization of a person as a source, while a shoeprints or fibers usually can serve to determine a type of shoe or cloth produced industrially. Databases have been created to assist these processes. For the handling of large masses of data, some criteria have to be respected: stability of the measured parameters, reproducibility and reliability of measurements, the precision, accuracy, and simplicity, the costs, time and availability of the methods, and finally the efficiency of screening systems needed to retrieve pertinent information from the database. The results must then to be interpreted and integrated into the investigation [Ribaux, 2005].

In the case of a pen entry, ink is transferred more of less forcefully from the ballpoint pen (source) to the paper, and aging will then occur over time. The product remaining after this aging constitutes the physical evidence for the document expert trying to date a document. Ideally, the changes due to the aging are reproducible, and an aging curve is available for comparison. If the analytical results correspond to an ink *n* years old, the forensic scientist must then interpret the significance of this match.

It was Popper who introduced to science a theory of deduction that is to be contrasted with the theory of the induction. Popper stated that there is no truth or falsity, but hypotheses which tend to be true or false with a certain

probability. Scientists have to test the degree of veracity of hypotheses [Popper, 1973]. This is an essential principle for the forensic scientist, who will have to determine the strength of the evidence and consequently calculate the probability of a hypothesis related to the criminal event. The Bayesian approach considers causality in the interpretation of the effects (i.e. evidence) [Aitken and Taroni, 2004].

In most cases where the date of a document is contested, one has to look at the probability of the evidence (E; e.g. analytical results) given the ink entry has been made at a time t_1 compared to the probability of this same evidence given the ink entry has been written at a prior time t_2 (fig. 2.7).

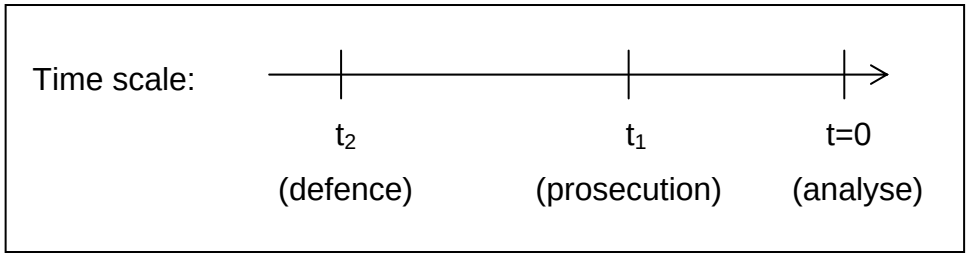


Figure 2.6 Time scale, where t_2 is the pretended date of the ink entry, t_1 is a posterior time, and t=0 is the time when the evidence is observed.

The prior probability of the hypotheses of the prosecution (H_p: entry was made at a posterior time t_1) and the defense (H_d: entry was made at time t_2) existing prior to the observation of the evidence *E*:

$$\text{Prior probability} = \frac{P(H_p)}{P(H_d)} \qquad \text{Eq. (2.7)}$$

is multiplied by a factor LR to obtain the posterior probability that accounts for the new evidence *E*:

$$\text{posterior probability} = LR \cdot \text{prior probability} \qquad \text{Eq. (2.8)}$$

The likelihood ratio (LR) is an indication on the strength of the evidence in supporting one of the hypotheses in Bayesian logic. It is defined by the probability of *E* given H_p is true divided by the probability of *E* given H_d is true:

$$LR = P(E/ H_p) / P(E/ H_d) \qquad \text{Eq. (2.9)}$$

With a value of LR = 3, for instance, it is three times more likely to observe the evidence *E* if the ink entry has been written at time t_1 (hypothesis H_p) than if it has been written at time t_2 (hypothesis H_d). A value of LR=1/3 means that it is three times more likely to observe the evidence *E* if the ink entry has been written at time t_2 than if it has been written at time t_1. The presence or absence of an infraction will be determined by the time at which the ink entry has been made. In this case, time is not a discrete variable that can take two values t_1 and t_2, but a continuous scale, and the scientists must deal with continuous data. Care must be exercised in checking the reproducibility and reliability of the data.

Travailler n’est pas une punition, travailler c’est respirer !

To work is not a punishment, to work is to breathe!

Le Corbusier

3 METHODS

3.1 Substances

The solvents: deionised water, ethanol, acetone, dichloromethane, and the internal standard (1,3 - benzodioxole - 5 – methanol) were purchased from Merck (Darmstadt, Germany), the solvent trifluoroethanol (TFE) was purchased from Fluka (Buchs, Switzerland).

The reference substances used were the pure dyes methyl violet “MV” (Fluka) and ethyl violet “EV” (Sigma-Aldrich). Other substances used as reference were pure ethoxyethoxyethanol “E” (b.p. 202℃, viscosity 4 cP) and dipropylene glycol “D” (isomers mixture; b.p. 230℃, viscosity 75 cP) from Sigma-Aldrich (Steinheim, Germany) and phenoxyethanol “P” (b.p. 247℃, viscosity 22 cP) from Riedel-de-Haën (Seelze, Germany). Phenoxyethoxyethanol “PE” was not available as a reference substance in Germany, and was studied exclusively in ballpoint ink mixture for our tests.

Eighteen blue ballpoint pens were purchased in different public outlets in Germany:

1a - BIC® (Cristal™ medium blue, France)
1b - BIC® (SOFTfeel Jumbo, medium blue, France)
2 - Caran d'Ache® M (Goliath 8422, medium blue, Switzerland)
3 - A.T.Cross® M (Lincoln RI02865, medium blue, USA)
4 - Diplomat® Spacetec M (Gas pressure, Medium blue, Germany)
5 - Faber-Castell® M (Graf, DIN 16554/2. 149741, Germany)
6 - Herlitz® (Medium blue, Germany)
7 - Lamy® M16 (ISO 12757-2 H, Medium blue, Germany)
8 - Lamy® M21 (ISO 12757-2 D, Medium blue, Germany)
9 - Mont-Blanc® (Medium blue, France)
10 - Parker® (Medium blue, ISO 12757-2, England).

11 - Pelikan® 337 M (DIN 16554/2, Medium blue, Germany)
12 - Pilot® RFT-4-F (Fine blue, Japan)
13 - Schneider Express® 75 M (ISO 12757-2 A2 ss, Medium blue, Germany)
14 - Shaeffer® M (Medium blue, USA)
15 - Staedler® 430 M (ISO 12757, Medium blue, UK)
16-Tombow® (ISO 12757, Medium blue, Japan)
17-Watermann® Std. Max.M (Medium blue, USA)

Four additional blue ballpoint pens from unknown manufacturers were obtained as advertising materials distributed by Burles Industries, Licher Beer, Ainea AG and PE SCIEX. The German Federal Bureau Criminal Investigation (Bundeskriminalamt, BKA, Wiesbaden) provided nine blue and one black ballpoint pens, and also supplied standard substances and ink from one of the blue and the black pen. Moreover, ink entries drawn on paper and held in a file (14 years, 4 years, 3 months old) were furnished by the BKA for five of the blue ballpoint pens.

The ballpoint entries were made on multifunction bright-white, wood and chlorine free paper from Igepa Plus printer paper (80 g/m^2, DINA4, nr. 806 A 80, Reinbeck, Germany). Subsequent analyses were carried out, either after dissolving the pure dyes in ethanol or dichloromethane, or directly from the sheet of paper.

3.2 Materials

For routine preparation, Eppendorf cups and pipettes (Eppendorf AG, Hamburg, Germany) were used. Dichloromethane extraction was performed in 50 µl glass micro inserts held in 1.5 ml threaded short amber glass vials. which were closed with silicone screw caps coated with PTFE (VWR, Darmstadt, Germany).

The LDI and MALDI sample plates were “home-made” and coated with gold. Small carbon stickers were used to attach pieces of paper on the surface. A standard 10 µl Hamilton syringe (Bonaduz, Switzerland) was used for sample injection into the GC. ESI capillary tips MC-10N (ID ~2 µm) were purchased from MasCom GmbH (Bremen, Germany).

An Heraeus oven (220V, 52 A, 50Hz, 1.14kW) set to a temperature of 100℃ was used to heat the samples. A high-pressure xenon lamp (Leitz GmbH, Wetzlar, Germany, 220/240V, 50Hz, XBO/CSX 450W) was selected for samples exposure to light, because the xenon lamp emission covers all wavelengths from 250 nm to more than 1000 nm and yields irradiance with sufficiently high fluence rates over the entire range of wavelengths (Table 3.1). Samples were positioned at a distance of 17 cm from the light source.

RANGE (nm): λ_1 to λ_2	**Irradiance (%): 200 to 2500nm**
<250	0.2
250-280	0.56
280-320	1.4
320-350	1.5
350-400	3.2
400-450	3.6
450-500	4.1
500-550	3.6
550-600	3.5
600-650	3.4
650-700	3.4
700-800	6.8
800-900	17.1
900-1000	17.9
1000-1100	5.5
1100-1500	10.7
1500-2000	8.1
2000-2500	5.4

Table 3.1 Percent spectral irradiance from the xenon high-pressure lamp as a function of the wavelength

The following Schott Glaswerke filters (40 x 40 mm^2, 2 mm thickness) were used to measure the influence of the wavelength on dye degradation: GG475 with transmission over 475 nm (yellow) and RG5 with transmission over 650

nm (red); in addition, a UV band pass filter SB-300-F from Laser Components GmbH (25.4 mm diameter, 5 mm thickness) was employed. The transmission curves are shown in Figure 4.17 a.

The photon flux was measured with two silicon photodiode from United Detector Technology, the first one (PIN-10DFP) producing a flat spectral response of 1.5 A/W between 400 to 1000 nm, and the second one (UDT-UV100, UV enhanced) offering spectral response between 200 to 1000nm (Figure 3.3). The first of these detectors was used to measure visible light. A grey filter NG5 (transmission de 20 %, 50 x 50 mm, 3 mm thickness) was used to attenuate the xenon lamp response. The second detector was used to measure UV light with a solar blind filter SB-300-F from Laser Components GmbH used to cut off the visible light (transmission of 40 % between 220 and 360 nm).

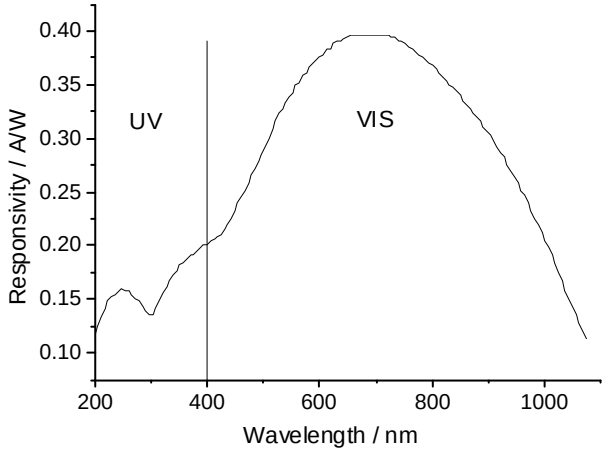


Figure 3.3 Responsivity in A/W as a function of the wavelength of the UV enhanced photodiode (UDT-UV100).

The relative numbers of photons per surface area and time unit were continuously measured in volts after being amplified by a factor of 10^4 V/A for the visible range, and of 10^6 V/A for the UV range, and then recorded with a high performance multifunctional PCI Adapter (PCI-1002L) with 32 SE/16D Analog Inputs, 12-bit ADC, 110 kHz, 16 DI, 16 DO and a timer from IPC2U (Industrial Personal Computer 2U GmbH, Langenhagen, Germany). The same card was used to record the temperature and humidity measured with two

universal thermo-hygrometer modules UTH 100 (ELV® Elektronik AG, Leer, Germany). The data were then processed using a program written by Alfons Hester (Analytical Chemistry, JLU Giessen, Germany).

3.3 Instruments

3.3.1 Aladim II (Advanced Laser Desorption Ionisation Mass Spectrometer)

Mass spectral analyses of chemical degradation of the pure dyes and of ink entries on paper were conducted on a laboratory-built MALDI/LDI Reflector Time-of-flight (TOF) mass spectrometer [Budzikiewicz, 1998]. Desorption and ionisation were performed with a pulsed nitrogen laser (337 nm, 3 ns, ~20 µm focus diameter). Delayed extraction was used, and only the positive ions were recorded. Mass spectra were recorded by summing the responses of 100 individual laser pulses; the typical mass resolving power was between 3000 and 6000. The laser irradiance was regulated with an attenuator between the laser and the sample (Fig. 3.4), so that it was possible to find the operative threshold irradiance at which a sample adequately desorbed and ionised. The following MS spectra were recorded for each point of a given aging curve:

Pure MV / EV : Degradation values were averaged from 10 summed spectra acquired from each of 1 to 4 sample spots.

BIC® strokes: Degradation values were averaged from 4 to 10 summed spectra acquired along 2 to 3 strokes.

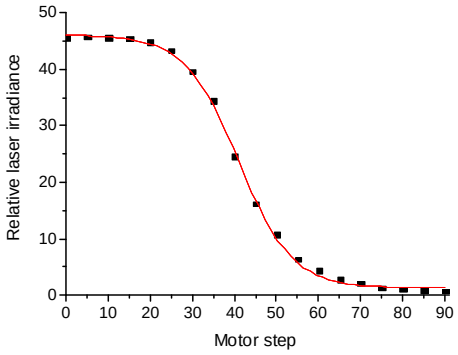


Figure 3.4 Relative laser irradiance as a function of step motor setting. An attenuator consisting of a dichroic quartz plate was placed between laser and sample , and its angle attenuating the laser irradiance transmission was adjusted by step motor. Typical MALDI and LDI mass measurements are made between steps 60 to 90.

3.3.2 Finnigan MAT4500 (Gas Chromatograph / Mass Spectrometer)

Quantitative analysis of the solvents was performed on a GC/MS MAT 4500 from Finnigan MAT GmbH (now Thermo Electron GmbH, Bremen). The instrument was equipped with the MASPEC Data System acquisition software (MSS - Mass Spectrometry Services Ltd., Manchester, England). The solvents were separated in a CP-Sil 8 CB low bleed / MS (corresponding to DB-5) capillary column from Varian (Chrompack, Middleburg, the Netherlands). The column was 60 m long and had an internal diameter of 0.25 mm and a film thickness of 0.25µm. The chromatographic elution was temperature programmed as follows: isothermal for 6 min at 50℃ , then at a rate of 10℃/min from 50 to 300℃, and finally isothermal for 5 min at 300℃. The carrier gas was helium (Messer Griesheim GmbH, Frankfurt, Germany) with a constant flow of ~3ml/min (at 30 psi / 2 bars). For the chromatographic separation, a solvent delay of 300s was chosen. For improved sensitivity, the sample was injected in the splitless mode, and the injector temperature was maintained at 200℃, which was sufficient to volatise all the substances of interest. The interface temperature was set to 250℃ in order to avoid reco ndensation. The MS part of

the GC/MS was a highly sensitive quadrupole instrument with a mass range up to 1000 u. Ions were formed by electron impact (EI) with a fixed electron energy of 80 eV. The temperature of the ionisation block was kept at 120℃. For qualitative analysis, the instrument was used in the SCAN or TIC (Total Ion Current monitoring) mode. Masses were scanned in the quadrupole from m/z 33 to 400 u at a sweep time of 0.95s. The resulting mass spectra were evaluated with the NIST database (MS Search Program Version 1.0, NIST, MSS Ldt. Manchester, England), which made it possible to identify the eluting substances. The obtained results were confirmed via GC/MS analysis of standard substances providing reference values for comparing the relative retention time and mass spectra. For better quantitative accuracy, the SIM (Selected Ion Monitoring) mode was employed, since it offers higher sensitivity. In this mode, lower quantities of solvents could be detected and quantified than in the TIC mode. For the purposes of ink solvents analysis, 15 particular ions corresponding to masses of 45, 59, 65, 72, 75 ,77, 89, 93, 94, 103, 104, 135, 138, 152 and 182 u were selected and monitored. These ions signals correspond to the internal standard and the solvents found in the selected ballpoint pens. All measurements were repeated at least three times.

3.3.3 Lamma 2000 (Laser Microprobe Mass Analyser)

For the determination of dyes and degradation products distribution along the surface area taken up by an ink stroke, a laboratory-built Laser Microprobe MALDI/LDI - TOF mass spectrometer with a pulsed nitrogen Laser (337 nm, 3 ns) was used. The instrument yields spatially resolved imaging analysis of the distribution of selected sample components on a surface [Spengler and Hubert, 2002]. The pulsed laser has a focus diameter of less than 1 µm and scans a surface of 10’000 μm^2 (10'000 laser shots). The analysis lasts about 30 minutes when recording at least four particular masses along the surface.

3.3.4 Finnigan LTQ FT™ (Linear Ion Trap Fourier Transform Ion Cyclotron Resonance Mass Spectrometer)

Electrospray Ionisation (ESI)-MS experiments of ballpoint dyes were performed on the Finnigan LTQ FT™ from Thermo Electron (Bremen, Germany). The segmented linear trap with radial ejection and dual detection system was used for LC/MS^n experiments. The instrument is equipped with high resolution Fourier Transform Ion Cyclotron Resonance mass analysis, but this feature was not used in the present work, since the Ion Trap gave sufficient mass resolution. The nanospray voltages ranged between 1 and 1.4 kV. With this instrument, the molecules are identified in terms of high resolution masses and fragmentation patterns.

3.3.5 Jasco UV/VIS Spectrophotometer

Absorption spectra of the dyes in solution were recorded with a V-550 Jasco UV/VIS Spectrophotometer. The measuring range extended from 190 to 900nm, and was scanned with a speed of 200 nm/min and a bandwidth of 2 nm. The Hellma® precision cells employed were made of Suprasil® quartz and offered a 10 mm light path.

3.3.6 Zeiss Microsspectrophotometer (MSP)

Absorption spectra of the ink entries on paper were performed with a Zeiss Axioskop instrument (Zürich, Switzerland) using an Epilon – Neofluar objective 20x / 0.50 HD. Light reflected between 380 and 780 nm was analysed with 5 nm steps. Optic brighteners contained in the paper altered the result in the UV range. Ten measurements per ink entries were averaged. This instrument was located in the Institut de Police Scientifique at the University of Lausanne in Switzerland.

3.3.7 CAMAG System (High Performance Thin-Layer Chromatography)

An HPTLC silica gel plate 60 from Merck (Darmstadt, Germany) and two CAMAG horizontal developing chambers (Muttenz, Switzerland) were used at the Institut de Police Scientifique (University of Lausanne, Switzerland) for chromatographic separation of dyes and pigments in ink entries. A CAMAG Linomat IV instrument offered semi-automatic sample application for qualitative and quantitative analyses. Densitometric comparison was carried out on a CAMAG TLC Scanner 3 by measurements of reflection in absorbance mode at a wavelength of 590 nm. Spectra recording, peak detection and semi-quantitative data were calculated by winCATS software. Twenty ballpoint pen entries were analyzed simultaneously with a standard spot of methyl violet on each plate. Analysis composed of the sample application (being the most sensitive operation), the separation and the densitometric evaluation, lasted about six hours.

3.3.8 Projectina Docucenter 4500

A Docucenter 4500 spectral comparator (projectina AG, Heerbrugg, Switzerland) that usually is used for examining documents in forensic laboratories was employed for spectral comparison of ink entries in the visible and infrared range at the German Customs Investigation Services (Zollkriminalamt, Cologne, Germany).

3.3.9 Mettler Toledo Microbalance

For weighting experiments, an AX26 Comparator microbalance (Mettler Toledo, Greifensee, Switzerland) was used in the microgram range.

3.3.10 Olympus Microscope

A Olympus® BX41 microscope working with transmitted light and attached with a TH4-200 reflected light source (Hamburg, Germany) was used for enhanced observation of the ink strokes. The software analySIS® (Soft Imaging System, Münster, Germany) was installed to record pictures.

3.4 Experiments

3.4.1 Dyes

Sample preparation

The reference substance for LDI was prepared by dissolving ~1µg/ml in ethanol. A 0.5 µl aliquot of this solution was deposited on a gold sample plate and analysed. For MALDI, the DHB matrix was prepared at a concentration of 10 mg/ml in a solution of water : ethanol (3 : 2), then 1 µl of this matrix solution was mixed with 0.5 µl of the dye solution on the sample plate giving an optimum molar ratio of about 1 : 1000. For spectroscopic measurements, pure or mixed dyes were dissolved in ethanol or water (0.01 to 0.05 µg / ml).

For the analysis of ballpoint pen entries, strokes were drawn with the pens on a sheet of paper with the help of a ruler while taking care to apply similar pressure and obtain comparable stroke quality. Preparation details depended on the type of analysis to be performed:

Extraction (LDI): Ink entries about 2 cm long were extracted with ethanol, TFE, phenoxyethanol and BIC® mix (ethoxyethoxyethanol : dipropylene glycol, 1 : 2) during 10 min at 60°C. T he BIC® mix is composed of the two solvents found in the BIC®

ballpoint pen used in the present work [Weyermann et al., 2003].

Extraction (TLC): Ink entries about 1 cm long were extracted with 30 µl methanol and kept in the dark during 24 hours at room temperature. Two spots of 5 µl and 2.5 µl were deposited for each analysis.

On paper (LDI): Three ink entries about 5 cm long were made once a month for each pen, and then exposed to diverse treatments or natural aging. For analysis on paper, small paper pieces measuring about 5 x 8 mm bearing 2-3 strokes running parallel to the long edge, were cut, glued to a metallic sample holder with a carbon tape, and introduced into the MS.

Composition

Standard dye powders from one blue and one black ballpoint pen were analyzed by LDI-MS and UV/VIS spectrophotometry. In addition, the potential of LDI-MS for the identification of dyes directly from ballpoint ink entries on paper was tested. Mass spectra of thirty blue pens were evaluated and compared to the results obtained by standard forensic methods: spectral comparison (analysis performed by Helmut Schwank from the ZKA of Cologne, Germany) and HPTLC (analysis performed by IPS of Lausanne University, Switzerland). Two sequential separations with freshly prepared separation systems were carried out by Raymond Marquis from the IPS: with mixture 1 (1-butanol : 2-propanol : acetic acid = 20 : 10 : 1) and with mixture 2 (1-butanol : ethanol : water : acetic acid = 150 : 30 : 39 : 4.5).

Influence of the method on the dye degradation

Dye degradation due to the effect of laser irradiation was studied. Since excessive laser intensity can cause dye fragmentation, the influence of the ionization conditions was tested. Thus, for both LDI and MALDI, the laser fluence was varied so as to find a setting that provided adequate sensitivity for analysis of the pure dyes. Then the experiment was carried out on BIC® ballpoint strokes on paper.

The influence of the sample preparation technique was evaluated by comparing MALDI-MS spectra of extracted ballpoint strokes in ethanol, acetone, TFE, phenoxyethanol, and BIC® mix with LDI and MALDI spectra of the strokes on paper. Following these experiments, the best ionisation method involving a minimum of preparation steps that could alter the sample was chosen.

Artificial Aging

The influence of heat and light on the aging of the reference substance was evaluated by depositing pure MV dye solution on two gold plates. The first plate was stored in the oven during 336 hours at 100°C, a nd the second plate was exposed to xenon light during 6 hours. MALDI measurements were carried out every 30 to 60 min. Beyond six hours only two further measurements were performed with the heated sample (at time t=7 and t=14 days).

In a second experiment, about 0.01 mg/ml of pure MV was dissolved in water or ethanol, and exposed to xenon light for several hours to determine the influence of solvents. Degradation of the dye was studied by UV/VIS spectrophotometry and MALDI-MS up to the time at which the dye signals had completely disappeared. Measurements were taken every hour. For MALDI-MS analysis, the DHB matrix was prepared at a concentration of 10 mg/ml in a solution of water : ethanol (3 : 2), 1 µl of this matrix solution was then mixed with an aliquot of 0.5 µl of the dye solution on the sample plate giving an optimum molar ratio of about 1 : 1000 for analysis. As the solvents evaporated during the experiment, a second illumination experiment was carried out with volume

adjustment in order to determine the kinetics of fading. After complete disappearance of the dye signal, additional GC/MS and ESI-MS analyses were carried out in an attempt to detect degradation products not revealed by LDI-MS as an aid for determining the degradation pathways.

Subsequently, experiments were carried out with blue BIC® ballpoint entries on paper, by storing them in an oven at 100℃ or ex posing them to xenon light during 50 hours. A reference sample was held in a dark box. LDI measurements were carried out every 5 hours.

The influence of humidity was checked by storing blue BIC® ballpoint strokes on paper in two tightly sealed quartz cells exposed to xenon light during 9 hours. One cell was dry, and the other contained 2 ml water beneath the paper samples bearing the strokes. The relative humidity of the cell containing water was nearly saturated (100%). The LDI spectra were recorded every 1 to 1.5 hours.

For an exploration of the effect of wavelength on dye degradation, the absorption curves of methyl violet and ethyl violet were measured with the spectrophotometer. Three filters were chosen while considering the wavelengths of absorption of the dyes, and placed between the xenon light source and blue BIC® ballpoint strokes on paper. Exposure was maintained over a period of 9 hours. A reference sample without filter was recorded, and spectra were measured every 1 to 1.5 hours. The flow of photons striking unit surface area of the sample was measured with two photodiodes.

Natural aging

For natural aging, two batches of ballpoint ink entries were prepared every month during one year with seventeen ballpoints each. One batch was stored as a reference in the dark inside of a drawer, and the other was attached to a window pointing northwest for its exposure to natural daylight through the glass. Both the reference and the light exposed sample were kept in the same room. There were no structures blocking the light in front of the window. The window was an iplus neutral R (Interpane Gmbh & Co) with two float glass panels (the

inside panel was coated). 76% of the incident light, but only 13% of the UV between 320 to 400 nm were transmitted through the double panes. The heat transmission coefficient has a value of 1.2 W/m^2K. The degradation experienced by BIC®, Herlitz, and Parker ink entry samples aged in winter and/or in summer was compared.

Line crossing

A new method was developed for determining the crossing sequences of heterogeneous crossings of ballpoint pen and printers lines on paper. Using Scanning Microprobe LDI-MS allows the imaging of the concentration profiles by scanning the surface taken up by the intersection. The crossings consisted of BIC® black ballpoint pen entries above and beneath lines generated by two printers, an HP Laserjet and an HP Deskjet printer. Small pieces of white paper bearing the line crossings were cut and mounted on a sample plate for scanning microprobe LDI-MS analysis.

3.4.2 Solvents

Sample preparation

For the acquisition of calibration curves for quantification, pure solvents were dissolved in dichloromethane (DCM) in concentrations of 0.0005, 0.001, 0.0025, 0.005, 0.0075, 0.01, 0.05 and 0.1 mg/ml, together with 0.0227 mg/ml of an internal standard (IS). In addition, the derivatisation of ballpoint reference solvents was tested with 1-naphtyl isocyanat and anthracene-9-carboxylic acid, so that less volatile derivatives of these solvents could then be analysed by MALDI-MS.

Ballpoint pen entries were drawn on paper with the help of a ruler and stored in a cabinet at room temperature. The entries were about 0.5 mm wide and 50 mm long. Fresh ballpoint pen entries were kept in the laboratory at constant

temperature, while older strokes (older than one month) were kept in an office that had no air conditioning. The standard extraction procedure involved cutting ballpoint pen entries of about 1 cm from the paper sheet in the shape of rectangular stripes measuring 10 x 2 mm, and placing them into a small vial. The solvents were extracted during 10 minutes in an ultrasonic bath in 10ul DCM with an IS concentration of 0.0227 mg/ml. A 2 µl aliquot from the extraction mixture was then injected in the splitless mode on the GC column. For paper blank and diffusion measurements, pieces of paper having identical dimensions were cut, and extraction carried out following the same procedure. For lateral diffusion experiments, stripes of paper running parallel to the stroke were cut out at different distances from the centre of the stroke.

Composition

Standard solvent mixtures and the pens for one blue and one black ballpoint ink were obtained from the BKA (Germany, Wiesbaden). They were analyzed by GC/MS together with the ink entries of thirty blue ballpoint pens.

Drying of pure solvents

The rates of evaporation of the pure solvents: ethoxethoxyethanol (E), dipropylene glycol (D) and phenoxyethanol (P), were determined, on one hand by weighting the residual amount from 400 µl of solvent that had been placed into a small plastic container having a surface area of 9 cm^2, and on the other hand, by applying 10 µl of solvent to a piece of paper and weighting the paper every hour. The influence of solvent volume on the evaporation was evaluated with 10, 20, 40, 60 and 100 µl of E on paper samples. Because of the nature of paper, weight measurements have relatively high error and will only be indicative of the real evaporation rates on paper.

Drying of ink

For the determination of the initial concentration of solvents in the ballpoint pens, the cartridge of a Parker® ballpoint pen was opened and the solvents quantified by GC/MS with the procedure mentioned. The experiment was repeated three times, and the mean weight percentage of solvent in the ink calculated. The mean weight of 1 cm ballpoint pen ink entries was determined by measuring six times the following parameters and then calculating the average values:

- weight difference of the pen before and after writing 20 entries of 5 cm length with it,
- weight difference of a piece of paper before and after writing 20 entries of 5 cm length on it.

As a test of reproducibility of the results for time t = 0 (i.e. just after the strokes were made), the procedure was repeated on different days.

On the other hand, the changes occurring in the quantity of solvent as functions of the time were determined for entries older than one and a half year. For each point in time, three entries were extracted and analysed by GC/MS with the procedure mentioned.

Once the ink has been applied to the paper, the solvents will diffuse and migrate away from the stroke through the paper until an equilibrium distribution is reached. To quantify this phenomenon, solvent extraction was performed from rectangular stripes of paper cut at distances of 2, 4, 6 and 8 mm parallel to the stroke, as mentioned above. In this way most of the paper area reached by lateral diffusion was covered by the analysis.

During the above tests, the importance of an additional process relevant to solvent losses from strokes was recognised. This process is a migration of the solvents from one sheet of paper to another one by solvent mass transport perpendicular to the paper surface. For quantitative determination of this solvent migration, blank sheets of paper were placed on top of or underneath a sheet of

paper having fresh ink stroke applied to it. The contact was made without any additional pressure, and lasted 15 minutes. The following arrangements were tested in order to understand the extent and importance of such processes:

- a blank paper was placed *on top* of the paper with the fresh ink entry directly *after* the application of the stroke,
- a blank paper was placed *underneath* the paper with the fresh ink entry *during* the application of the stroke,
- a blank paper was placed *on top* of the paper with the ink entry *30 minutes after* the application of the stroke

The influence of the temperature on the drying process was determined with the aim of evaluating the artificial aging method proposed in the literature [Aginsky, 1996]. At first, a BIC® ballpoint pen entry was analysed after 20 minutes storage in an oven at 100°C. Then Parker entries have been aged in an oven maintained at 60°C for 0.5, 1, 5, 24 and 168 days. Comparison with sample age at room temperature (20°C) was carried out.

3.4.3 Tests with entries of known dates

The department of physical and chemical document examinations in the BKA (Wiesbaden, Germany) is in possession of a small collection of blue ballpoint pens. Entries have been drawn with these pens over a period of 14 years, and were naturally aged by storage in a file folder in their labs. They provided three entries for each of five inks, as well as the source ballpoint pens. New entries at time t=0 were drawn to determine the initial composition of the inks. LDI-MS and GC/MS were performed and results were evaluated in order to determine whether they were consistent with their known age (time between the date of entry and the analysis). Further entries were produced, aged for 2 to 8 weeks and analysed by GC/MS in order to compare solvent quantitative results. The papers substrates used were different for all the entries obtained.

Tempus fugit

Ovid

4 RESULTS AND DISCUSSION

4.1 Dyes

MALDI mass spectra of the pure dye MV are characterised by the presence of the molecular ions M^+= 372.2 u. In an earlier paper [Weyermann et al., 2003], the author detected the typical degradation of MV under the effect of light, which is characterised by a progressive loss of CH_2 groups (see Figure 4.1).

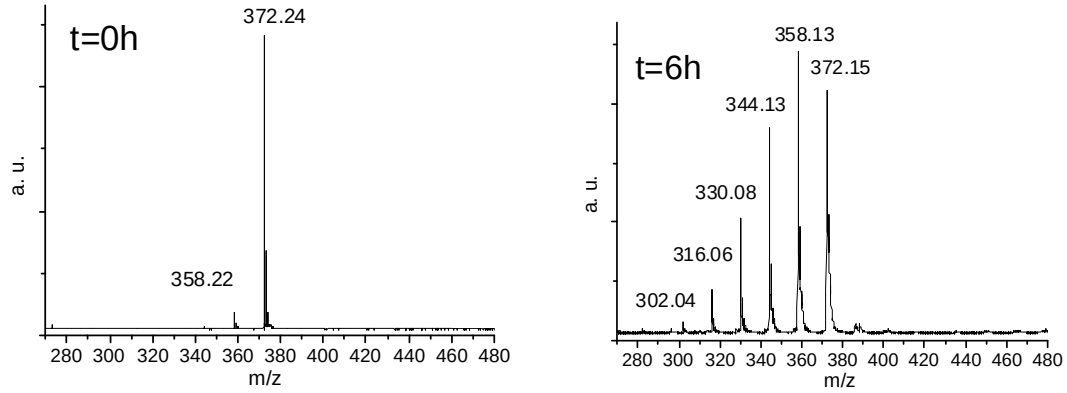


Figure 4.1 MALDI-MS Spectra of MV (methyl violet; M^+=372.2 u) dissolved in ethanol (~1mg/ml) prior to and after 6 hours of exposure to the light of a high-pressure xenon lamp: signals from five of six degradation products of MV (Δ=14 u) are present. The sixth degradation product with m/z = 288.0 u was observed after longer exposure.

Consequently, MV has six degradation products (Δ=14 u) with m/z = 358.1 u, 344.1 u, 330.1 u, 316.1 u and 302.0 u, and 288.0 u which are easily detected by MALDI-MS. This degradation is readily quantified by the Relative Peak Area (RPA) in % defined in the author's earlier work, as follows:

$$RPA_i = \frac{A_i}{A_{tot}} \cdot 100 \qquad \text{Eq. (4.1)}$$

where A_i is the area of the signal at m/z = *i* and A_{tot} is the total area of all signals (molecular ion and degradation products) of the dye. With this definition, it is then possible to define aging curves of RPA_i as a function of time. For example, the relations:

- $RPA_{372} = A_{372}/(A_{372}+ A_{358} + A_{344} + A_{330} + A_{316} + A_{302} + A_{288})$
- $RPA_{358}=A_{358}/(A_{372}+ A_{358} + A_{344} + A_{330} + A_{316} + A_{302} + A_{288})$

characterise the degradation of the molecular ion with m/z = 372.2 u as well as the production of the first degradation product with m/z =358.2 u. The MV powder that had been purchased already contains traces of the degradation products M^+= 358.2 u and M^+= 344.2 u, in addition to the pure dye M^+= 372.2 u.

The above definition was used to follow the aging of MV from several ballpoint pen entries on paper. Some inks contain additional dyes or pigments giving signals that may interfere. Some ballpoint pens used for the experiments actually contained other dyes in addition to MV, and signals from their degradation products may have overlapped with MV signals. In such cases, instead of A_{tot}, only the products clearly related to MV were taken into account when calculating the RPA values. For example, the dye ethyl violet (EV) has a structure very similar to that of MV, the methyl groups are replaced by ethyl groups. The molecular ions of EV can be described by M^+= 456.3 u, while the degradation products yield MALDI mass signal (Δ=28 u) at m/z = 428.2 u, 400.2 u, 372.2 u, 344.1 u, 316.1 u, and 288.0 u. Between these peaks, EV yields another set of less intense signals (Δ=14 u) at m/z = 442.2 u, 414.2 u, 386.2 u, 358.1 u, 330.2 u and 302.1 u (Figure 4.2).

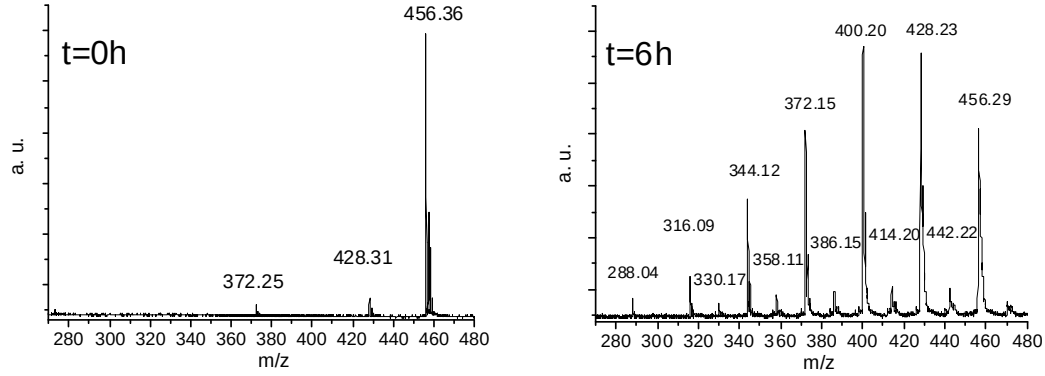


Figure 4.2 MALDI-MS Spectra of EV (ethyl violet; M^+=456.4 u) dissolved in ethanol (~1mg/ml) prior to and after 6 hours of exposure to the light of a high-pressure xenon lamp: strong signals from six degradation products (Δ=28 u) and, between them, another set of five weaker signals from six degradation products (Δ=14 u).

A BIC® ballpoint pen used for some experiments contained both MV and EV. Therefore, degradation product signals of EV overlapped with MV signals, hence the most important RPA values used to characterise strong degradation stages (where the signals from degradation products of the two dyes overlapped) were modified to read:

- $RPA_{456} = A_{456}/(A_{456}+A_{442}+A_{428}+A_{414}+A_{400})$
- $RPA_{428} = A_{428}/(A_{456}+A_{442}+A_{428}+A_{414}+A_{400})$
- $RPA_{400} = A_{400}/(A_{456}+A_{442}+A_{428}+A_{414}+A_{400})$

These equations characterise degradation of the molecular ion with m/z = 456.4 u, as well as production of the second and fourth degradation product (m/z = 428.3 u and 400.3 u) of EV.

The reproducibility of the results was found to be better in LDI measurements of strokes on paper than in measurements of the pure dyes. The mean standard deviations of the RPA values were calculated and used as error bars in the plots.

Composition

Reference substances

Four different dye standards and two ink pastes obtained from the BKA were analysed by UV/VIS spectrometry and LDI-MS:

- Parisviolett, Viktoriablau and Astrablau (blue ink mixture)
- Neptun Schwarz (black ink mixture)

The names give an indication of the dye contained in these mixtures, and MALDI mass spectra of the powders dissolved in ethanol (about 0.01 mg/ml) confirmed the identification. Parisviolett (Paris violet) contained the dye methyl violet (m/z = 372, 358 and 344 u), Viktoriablau (Victoria blue) contained the dye basic blue 26 (m/z= 470, 456 and 442 u), and Astrablau (Astra blue) contained a derivative of copper phthalocyanine (m/z= 1233, 1149, 1069, 984, 904, 820, 740, 656, 574 u), while Neptun Schwarz (Neptune black) contained the two dyes of methyl violet (m/z = 372 and 358 u). Metanil yellow is an anionic dye and was not detected in the positive ion mode spectra, but is known to be present in the Neptunschwarz mixture. The BKA (Wiesbaden, Germany) and the IPS (Lausanne, Switzerland) identified its presence (375 g/mol) by HPLC and HPTLC respectively. The mass spectra of the blue ink revealed a mixture of MV, Basic Blue 26, and the derivative of copper phthalocyanine, while spectra of the black ink only showed MV signals.

UV/VIS spectrometry of the ethanol solutions reveals absorption maxima for Parisviolett (580, 302, 262 and 208 nm), Viktoriablau (600, 346, 254 and 210 nm), Astrablau (668, 626, 344 and 218 nm) and Neptunschwarz (588, 420, 304, 250 and 208 nm). A superposition of the absorbance spectra of Parisviolett, Viktoriablau and Astrablau reflects the absorbance of the blue ink (λ_{max} = 662, 588, 352, 304 and 202 nm), while the absorbance of the mixture Neptunschwarz corresponds to that of the black ink (λ_{max} = 590, 418, 304, 252 and 208 nm). These results are illustrated in Figure 4.3.

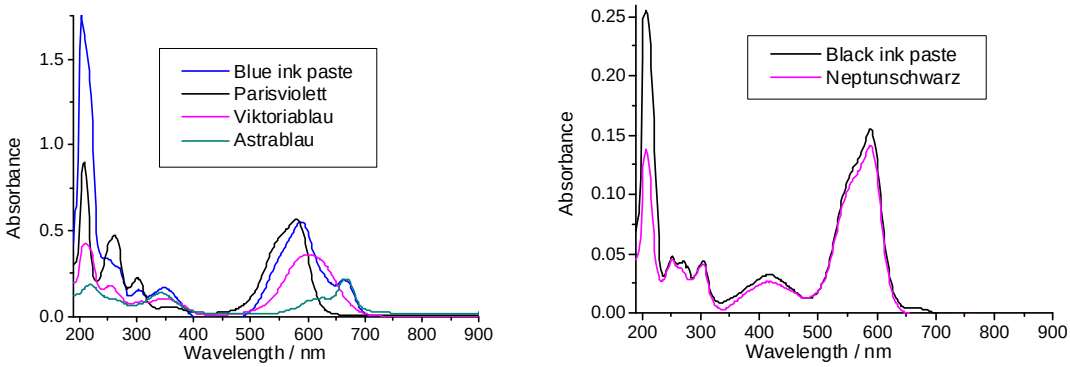


Figure 4.3 Absorption spectra of blue ink paste and its dye component mixtures (Parisviolett, Viktoriablau, Astrablau), as well as black ink paste and its dye component mixture (Neptunschwarz). The powders were dissolved to 0.01 mg/ml in ethanol for UV/VIS analysis.

Ballpoint pens

Ink entries made with the thirty-two ballpoint pens available for the present work were analyzed by LDI-MS. The results are summarized in Table 4.1. The following dyes were detected in the mass spectra of the 31 blue pens (reported in order of increasing m/z): methyl violet (MV, CI 42555, in 87% of the pens), basic blue 26 (BB26, CI 44045, 58% of the pens), Basic Blue 7 (BB7, CI 42595, 26% of the pens), Basic Blue 2 (BB2, CI 50240, 10% of the pens) and ethyl violet (EV, CI 42690, in 3% of the pens). Moreover, Pigment Blue 15 (PG15, 13% of the pens) was also recorded. The black ballpoint pen has the same cationic dye composition as the corresponding blue one (MV and BB26). For development of the black color, it probably contained soot as well as an anionic yellow dye that cannot be detected in the positive ion mode of the mass spectrometer. Also, 48% of the ballpoint pen inks gave rise to additional signals that could not be identified. Some signals probably were due to derivatives of copper phthalocyanine, but other methods have to be adduced in order to elucidate their exact structure.

No.	PEN	MV	EV	BB26	BB7	BB2	Others	TOT
1a	BIC	1	1	0	0	0	0	**2**
1b	BIC	1	0	0	0	0	0	**1**
2	Caran d'Ache	1	0	0	1	0	575, 594, 608 g/mol	**2**
3	A.T.Cross	1	0	1	0	0	580, 594, 608, 720 g/mol	**2**
4	Diplomat	0	0	1	1	1	512, 498, 720 g/mol	**3**
5	Faber-Castell	1	0	1	0	0	515 g/mol	**2**
6	Herlitz	1	0	0	0	0	515 g/mol	**1**
7	Lamy M16	1	0	1	0	0	515, 594, 608, 720, 770 g/mol	**2**
8	Lamy M21	1	0	1	0	0	515, 594, 608, 720, 770 g/mol	**2**
9	Mont-Blanc	0	0	1	1	0	176, 653, 720 g/mol	**2**
10	Parker	1	0	0	1	0	0	**2**
11	Pelikan	1	0	1	0	0	0	**2**
12	Pilot	1	0	0	1	0	0	**2**
13	Schneider Express	1	0	1	0	0	219, 241, 515, 608, 720, 770, 951 g/mol	**2**
14	Shaeffer	1	0	1	0	0	720 g/mol	**2**
15	Staedler	1	0	1	0	0	503 g/mol	**2**
16	Tombow	1	0	1	0	0	0	**2**
17	Watermann	1	0	0	1	0	0	**2**
A1	Burles	1	0	1	0	0	264, 594, 608, 720, 770 g/mol	**2**
A2	Licher Bier	1	0	0	0	0	0	**1**
A3	Ainea AG	1	0	0	0	0	0	**1**
A5	PE SCIEX	1	0	1	0	0	492, 575 g/mol	**2**
B1	BKA blue	1	0	1	0	0	252, 492, 580, 594, 608, 624, 727 g/mol	**2**
B2	*BKA black*	*1*	*1*	*0*	*0*	*0*	*0*	*2*
B3	1985 PG3	1	0	1	0	0	594, 608 g /mol	**2**
B4	303/103	0	0	0	1	1	0	**2**
B5	B4466	1	0	0	0	0	0	**1**
B6	303/401 B	1	0	1	0	0	0	**2**
B7	303/110B	0	0	0	1	1	498, 575, 736 g/mol	**2**
B8	303/701NB	1	0	1	0	0	498, 576 g/mol	**2**
B9	Ronsinco	1	0	1	0	0	512, 580, 594, 608 g/mol	**2**
B10	Stanis 372	1	0	0	0	0	0	**1**
%	**In blue pens**	**87**	**3**	**58**	**26**	**10**	**23**	

Table 4.1 Dye composition of 32 ballpoint pens determined by LDI-MS (1: present, 0: absent): the dye methyl violet (MV), basic blue 26 (BB26), Basic Blue 7 (BB7), Basic Blue 2 (BB2), and ethyl violet (EV) have been found in several ballpoint pens. Additional signals present in the mass spectra could not be identified.

The ballpoint ink compositions change from pen to pen, and from batch to batch, even within samples from the same company. We observed, for instance, that two blue ballpoints BIC® bought at an interval of a few months differed in dye compositions. From the selection of 31 blue ballpoint pens obtained from different companies, 28 (87%) contained MV as a mixture of the hexamethylated form (m/z =372.2 g/mol) and the pentamethylated form (m/z = 358.2 g/mol), while four did not contain any MV. It follows that at time zero, i.e. when the aging has not yet started, the RPA_{372} value can already be significantly below 100%. RPA_{372} values from the 32 ballpoint pens that were determined prior to aging ranged from 53% to 92%, hence the aging behaviours differ accordingly. In other words, the initial composition of an ink has to be known for a correct interpretation of mass spectra.

Subsequent analyses have been carried out with ballpoint ink entries containing MV, mainly from the BIC (1a); inks from BIC (1b), Herlitz (6), and Parker (10) were analyzed as well (B4, B5, B6, B7 and B8). As most of the ballpoint pens contained MV, the fading of this pure dye has been studied in particular. EV was present in the BIC (1a) ballpoint pen, and hence was studied to some extent. Its fading is very similar to that of MV.

Comparison with standard methods

Standard procedures used in the forensic examination of ink include optical methods such as spectral comparison at different excitation wavelength and HPTLC. They aim, not so much at the identification of a particular ink type but, rather, at the discrimination of ink entries in a document. Thus, revealing that an addition was made with a different pen may well lead to the conclusion of a falsification. The discrimination powers (DP) of the two methods were compared according to the following equation:

$$DP = \frac{\text{Number of discriminated pairs}}{\text{Number of possible pairs}} \qquad \text{Eq. (4.2)}$$

Where the number of pairs for a sample number of n equal:

$$\text{Number of pairs} = \frac{n \cdot (n-1)}{2} \qquad \text{Eq. (4.3)}$$

On the basis of the results provided for spectral comparison by the ZKA (Cologne, Germany) and for HPTLC by the IPS (Lausanne, Switzerland), 5 and 18 classes of ballpoint inks were, respectively, distinguished, while LDI-MS results led to 26 categories. Spectral comparison failed to discriminate the following 93 pairs of ballpoint pen entries:

- Herlitz, Burles, Licher, Ainea, PE Sciex, 303/701B, 303/401B, Stanis, 1985PG, BKA blue
- Faber-Castell, Lamy M16, Lamy M21, Pelikan, Schneider, Staedler, B4466
- Diplomat, Mont-Blanc, Pilot, Tombow, 101/103, Ronsinco
- Caran d'Ache, A.T. Cross, Schaeffer, 303/110B
- BIC1a, BIC1b, Parker, Watermann

Thirty-seven pairs of ballpoint pen entries were not discriminate by HPTLC:

- Faber-Castell, Lamy M16, Lamy M21, Pelikan, Schneider, 1985PG, Ronsinco, BKA blue
- B4466, Stanis, Ainea, Licher
- A.T. Cross and Schaeffer
- Parker and Watermann
- 303/701 NB and 303/401 B

And LDI-MS did not discriminate the following five pairs of ballpoint pen entries:

- BIC1b and Licher
- Lamy M16 and Lamy M21
- Pelikan and 303/401B

- B4466 and Stanis
- Parker and Watermann

As can be expected from the above exposed results, spectral comparison yielded the lowest DP (80%), while the DP is slightly higher for LDI-MS (99%) than for HPTLC (92%). The reason is mainly that two large groups of ballpoint entries were not differentiated by the latter technique: ink containing only MV (4 pens) and inks containing MV and BB26 (7 pens).

It is interesting to observe that the classifications obtained by the different methods were to some extend complementary. Also LDI-MS has the best discrimination power of the three methods, mass spectra of the BIC 1b ballpoint pen only showed the dye MV, while the spots on the HPTLC plate indicated at least two additional dyes (Fig. 4.4). One dye did not migrate on the HPTLC plate. However, it was visible as a light blue shade spot where the ink was apposed. It was possible to identify the presence of this dye in eight ballpoint inks by HPTLC, while it was not recorded in the LDI mass spectra. Anionic dyes were not detected by LDI-MS in the positive mode, while they were easily detected by HPTLC.

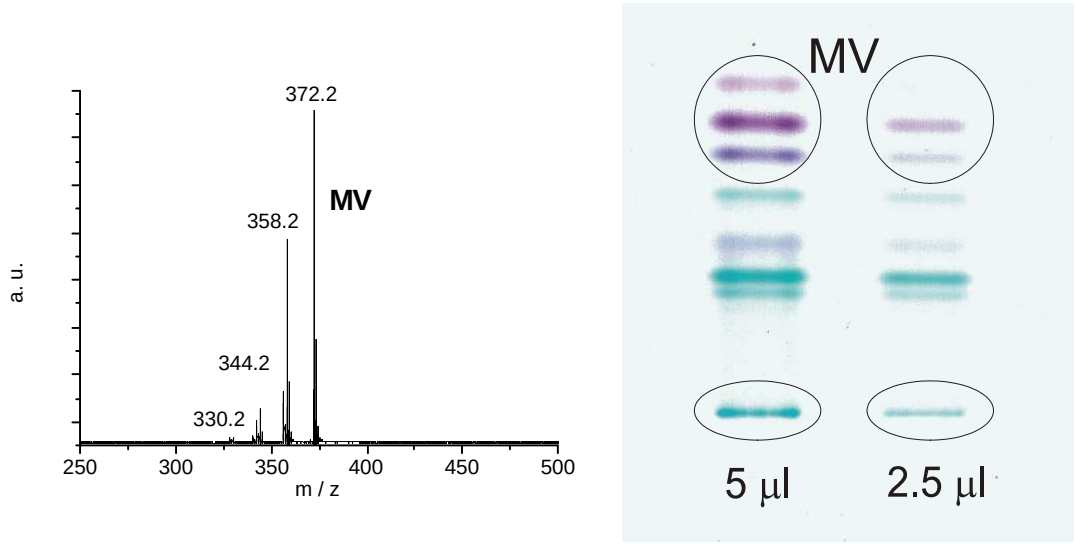


Figure 4.4 LDI mass spectra (to the right) and HPTLC plate (to the left) of the BIC 1b ballpoint pen. While only MV was detected by LDI-MS, at least two additional dyes were detected by HPTLC analysis. One dye did not migrate on the plate.

On the other hand, the presence of additional peaks in the mass spectra and the determination of the RPA values of the identified dyes, allow the LDI-MS technique to discriminate additional pairs. For example, the ballpoint pens Pelikan and 1985 PG3 were not discriminate by HPTLC, because their chromatography gave very similar results. The intensities of the peaks were different (Fig. 4.5), but it can be explained by slightly difference of the volumes apposed on the plate. They were discriminate by LDI-MS because of the large difference in the RPA value of the dye MV (82% for pen Pelikan against 53% for pen 1985 PG3). Moreover HPTLC was not able to separate the dyes MV and BB26, due to the fact that they had identical retention times. When one of these dyes was markedly more concentrate than the other, it was the difficult to identify both dyes (Fig. 4.5).

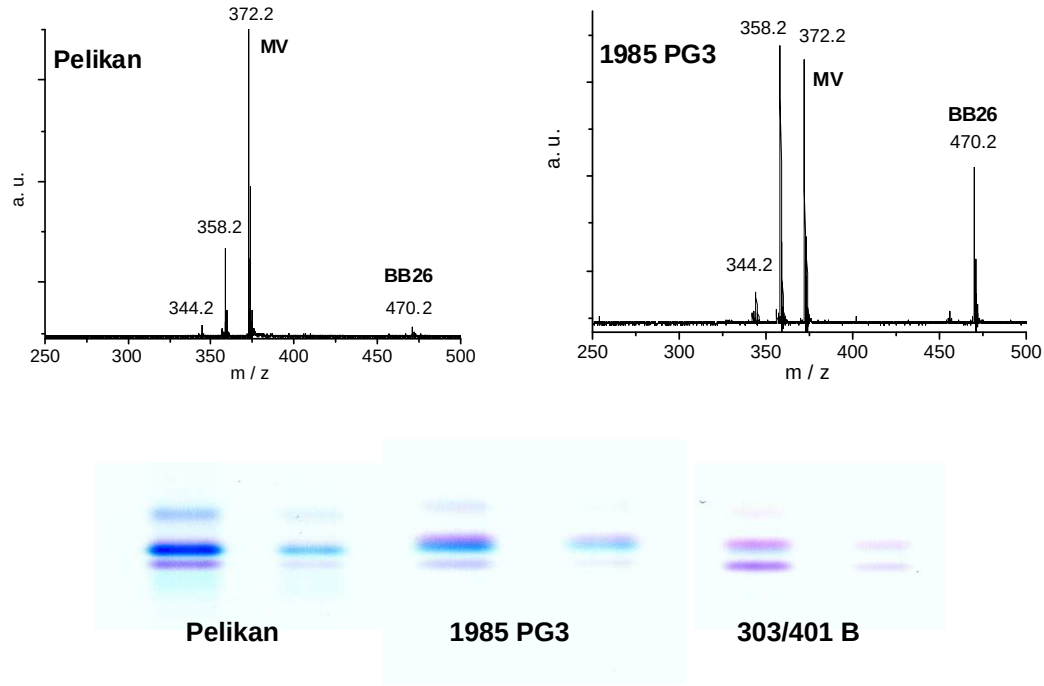


Figure 4.5 In the LDI-MS spectra (above) of the ink entries from ballpoint pens Pelikan and 1985 PG3, signals from the dyes MV and BB26 have been identified. On the TLC plate (below), ink entries of the ballpoint pens Pelikan, 1985 PG3 and 303/401 B spots from MV (violet) and BB26 (blue) have also been identified, but they are partly superposed because they did elute together. When one of the dyes is markedly more concentrate than the other, it is difficult to identify both dyes.

Four pairs of pens (Parker and Watermann, Lamy M16 and Lamy M21, Pelikan and 303/401 B, B4466 and Stanis 372,) were not discriminate by any of the methods used. This could eventually mean that their ink formulation was indeed identical; also this statement could not be confirmed. Actually pen Pelikan was made in England, while pen Watermann was made in USA, so it is difficult to assess if they could actually be from the same batch.

One of the major advantages of LDI-MS over HPTLC is a minimum sample preparation and destruction, as analysis is conducted directly on paper. The method is therefore very rapid compared to the long preparation and elution time needed for the chromatography. Moreover the mass spectra yield information about the structure of the dye and may assist in their identification. Finally, additional signals in the mass spectra (from pigments or additives) and the RPA definition used to calculate the ratio of a dye and its *N*-demethylated products allowed for an improved discrimination. On the other hand HPTLC detected signal from anionic dyes which were not present in the LDI mass spectra. The negative mode detection would probably resolve this problem, while an improvement in the preparation method (eventually by adding a matrix to the ink strokes on paper) may allow the detection of additional dyes or pigments in the mass spectra.

Influence of the method on dyes fading

Laser irradiance

For this experiment, samples of pure MV and EV deposited from an ethanol solution onto a gold target were analysed with MALDI. The samples were mixed with matrix directly on the plate. Measurements were started at a high fluence, which then was decreased stepwise down to the threshold fluence defined as the lowest laser irradiance at which a signal was obtained.

It was observed that the fragmentation of the molecular ions increased with the intensity of laser irradiance (RPA_{372} and RPA_{456} decreased) and the degradation product signals increased (RPA_{358}, RPA_{344}, etc. and RPA_{442}, RPA_{428}, RPA_{372}, etc., respectively, increased). The curves in Figure 4.6 show

stronger fragmentation of the molecular ion of EV as compared to MV. The threshold fluence at which a minimal signal was observed was not the operative fluence at which good measurements could be performed. Thus, RPA values increased with increasing irradiance fluence until the operative fluence was attained, and then decreased again, because fragments were produced during the desorption and ionisation process. Moreover, new fragments (such as RPA_{356}, RPA_{357}, RPA_{368}, RPA_{413}, RPA_{415}) appeared in the spectra at higher fluences, but were not taken into account in the RPA definition. A value of 1.5 was chosen for the ratio of operative to threshold fluence, which is equivalent to an operative fluence of $3 \cdot 10^3$ J/m^2.

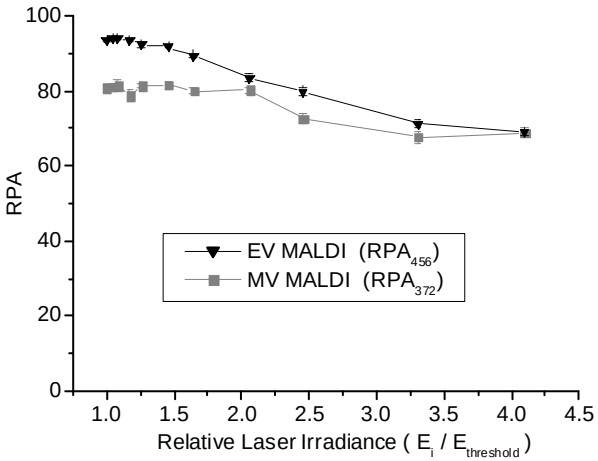


Figure 4.6 Fragmentation of MV and EV as a function of laser irradiance. The values of RPA_{372} and RPA_{456} which are the relative signal areas of the molecular ion (M^+=372.2 u and M^+=456.3 u) as well as a measure of the degradation of the dyes were calculated from the MALDI-MS spectra of MV and EV dissolved in ethanol (~1 mg/ml). High laser fluences promote degradation of the molecular ions, therefore, measurements had to be performed with fluence close to threshold. A relative value of 1.5 was chosen for the operative fluence in the measurements.

In the second part of this experiment, the influence of the laser fluence on fragmentation was tested for BIC® 1a ballpoint strokes on paper rather than in the pure dye solutions. Laser irradiance also provoked fragmentation of the dyes, and for this particular ink, the dye MV was found to be less stable to laser irradiance than the dye EV (figure 4.7). It was also observed that older samples generally exhibit higher threshold laser irradiances than samples freshly

prepared. This observation can be explained by solvent evaporation taking place after application of the ink to paper. Resins and dyes are expected then to form a more compact surface.

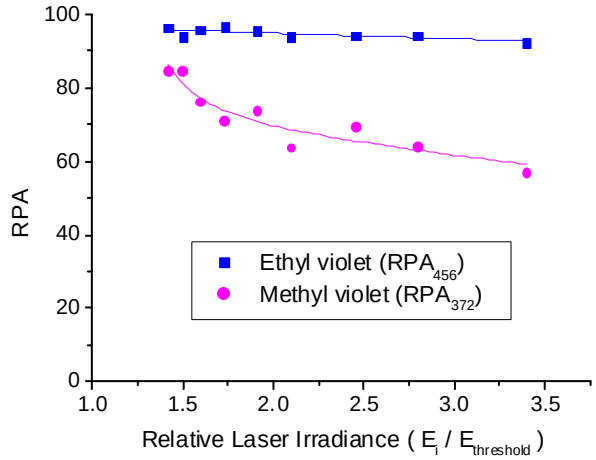


Figure 4.7 Fragmentation of MV (RPA_{372}) and (EV RPA_{456}) calculated as a function of the laser irradiance from the LDI-MS spectra of the BIC® 1a ballpoint pen. For this particular ink, the dye MV was found to be less stable to laser irradiance than the dye EV. A relative value of 1.5 was chosen for the operative fluence in the measurements.

Since the laser irradiance has a measurable influence on the results, it is important to analyse aged samples a fixed fluence in order to allow meaningful comparison. We have chosen to record our results at an operative fluence (relative value of 1.5) close to the threshold value of old inks.

Preparation method

A comparison between MALDI and LDI experiments was used to test the chemical influence of the matrix on the degradation of pure dyes dissolved in ethanol. The results show that the molecular ions of the dyes degraded already during the MALDI preparation and/or desorption / ionisation steps of the experiment. Both RPA_{372} and RPA_{456} were already significantly below 100% (Fig. 4.6).

In a further experiment, MALDI and LDI analyses of strokes directly on paper were compared with MALDI analyses of different extraction solutions of ballpoint strokes (Fig. 4.8). No solvent completely extracted all ink components, and the mass spectrometric signals were weaker than the ones obtained

directly from the strokes on paper. Extraction by ethanol, acetone, and TFE led to values below 60% for RPA_{372}, and below 40% for RPA_{456}.

Phenoxyethanol and the BIC mix (ethoxyethoxyethanol : dipropylene glycol, 1 : 2) are typical ballpoint pen compounds [Weyermann et al., 2003; Fortini, 2000; Lociciro et al., 2004]. They are good extraction solvents for ballpoint dyes, and led to a signal intensity of the molecular ions that was higher than that obtained with the other solvents. The values were between 70 and 80% for RPA_{372}, and between 50 and 60% for RPA_{456}. Nonetheless, even here the LDI and MALDI analyses of the strokes on paper still gave more intense signals with minimal degradation of the molecular ions of MV and EV: about 85% for RPA_{372}, and 80% for RPA_{456}. These results show clearly the influence of extraction and matrix sample preparation on the determination of the degradation. Since the dyes readily absorb the laser light, and are easily ionised, using a matrix in our case did not improve the measurements on ink strokes. Moreover, the matrix used is an acid that could chemically influence the organic dyes. Therefore, the author suggests that for best results, LDI analysis of the ballpoint strokes should be performed directly on paper, and close to threshold laser irradiance. This has the advantage of reducing to a minimum the sample preparation steps and associated alterations of the samples.

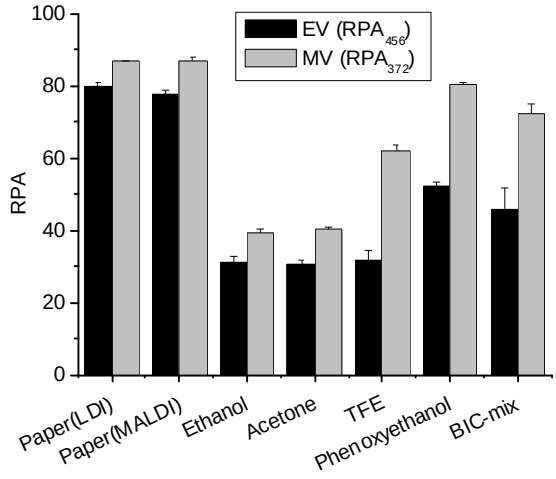


Figure 4.8 RPA_{456} (EV) and RPA_{372} (MV) values from LDI and MALDI mass spectra of BIC® ballpoint pen strokes on paper as well as from MALDI mass spectra of extracts of the paste from BIC® ballpoint pen entries (about 2 cm) in diverse solvents. The results with the minimal fragmentation were obtained for the measurements performed directly on the paper (high RPA values).

Fading of pure MV

Light and heat

In preliminary studies of the influence of light and heat on pure MV, it was confirmed that degradation of the dye strongly depends on the quality and duration of irradiation. After six hours of exposure to the xenon light, the RPA_{372} value calculated from the MALDI-MS spectra had decreased to 25% (Fig. 4.9). The influence of exposure to heat was found to be weaker, but heat also provoked degradation. After one week exposure, the RPA_{372} value calculated from the MALDI-MS spectra had dropped to about 40%. This indicates that degradation of the dyes proceed even in the absence of light.

The question of dye degradation upon very long exposure to heat, and its extent relative to that observed upon exposure to light, was not addressed in the present work. It was seen, though, that the aging curves appear to level off after longer times of exposures to light or heat, implying that the degradation process reaches a limit or is slowing down significantly.

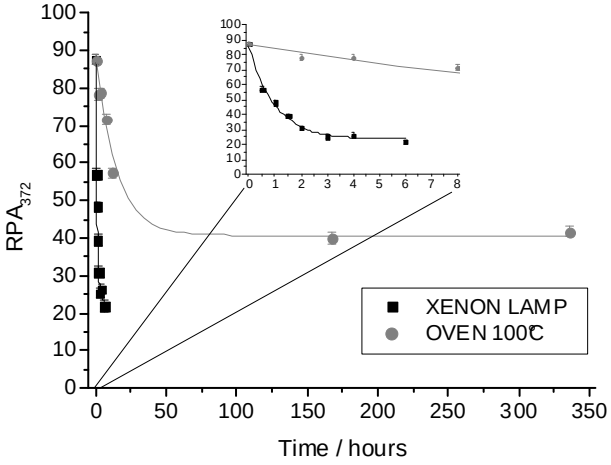


Figure 4.9 Comparison of the degradation of methyl violet (~1 mg/ml in ethanol) provoked by light from a high-pressure xenon lamp (d = 17cm) over a period of six hours, and by heat in an oven at 100℃ over a period of 336 hours (14 days). Upon exposure to light, the RPA_{372} values dropped from ~97% to ~25 % after 3 hours. The RPA_{372} value of sample stored in the oven had dropped to ~40% after 7 days treatment and remained roughly constant upon further exposure.

Solvents

For this experiment, aqueous and ethanol solutions of MV (0.01mg/ml) were exposed to light in spectroscopic quartz cells. Once an hour a UV/VIS-spectrum was recorded, and a 0.5 µl aliquot was taken for MALDI-MS analysis. Degradation was noticeably differently in the two solvents.

In water, both a decrease and a shift of the absorption maximum, corresponding to the degradation products mentioned above were observed by UV/VIS (Figure 4.10a). The disappearance of the MV signal and the consecutive formation and degradation of the degradation products was followed by MALDI analysis (Figure 4.10b). MV in water gave an absorption maximum at 590 nm and a large signal at m/z= 372 in the MALDI mass spectra. The maximum shifted to 580 nm after 12 hours exposure to xenon light (corresponding to formation of a peak at m/z = 358 u), then to 571 nm after 19 hours (corresponding to m/z = 344 u), to 566 nm after 26 hours (corresponding to m/z = 330 u), to 556 nm after 34 hours (corresponding to m/z = 316 u), to 548 nm after 38 hours (corresponding to m/z = 302 u) and finally to 540 nm after 42 hours (corresponding to m/z = 288 u). The strong absorption features and the MS signals disappeared from the spectra after about 54 hours.

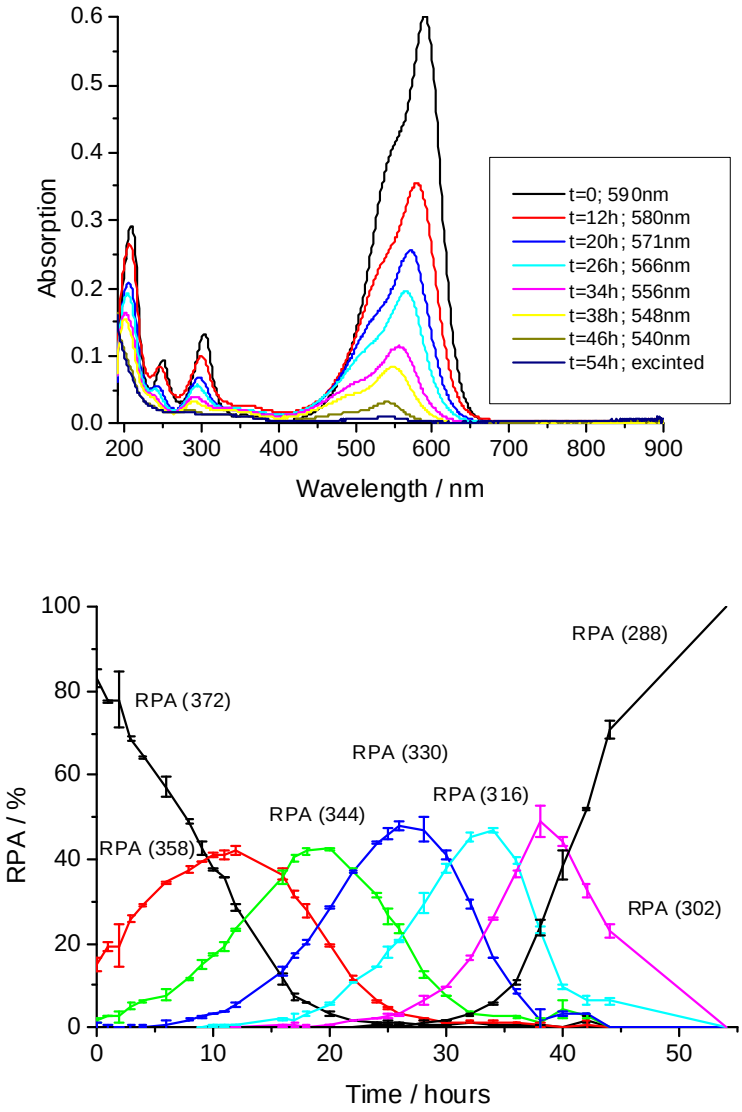


Figure 4.10 Exposure of an aqueous solution of MV (0.009 mg/ml) to xenon light (a) UV/VIS absorption spectra for different illumination times, (b) Relative Peak Area (RPA) values for different ionic species calculated from the MALDI mass spectra as a function of time [hours]. The RPA values describe degradation of the dye and production and further degradation dye fading products.

Quite differently, no shift was observed in the absorption maximum of MV in ethanol solution. In this case the maximum occurred at a wavelength of 588 nm (see Figure 4.11a) slightly different from that observed for absorption in water. This observation is an indication for different interactions of the substance with the solvents, probably resulting in a slightly different electronic configuration, and hence a different absorption profile. Absorption decreased upon

illumination, and a semi reversible change (also called phototropy) was detected in the UV range: absorption at 202 nm increased during exposure to light, and slightly decreased again after a certain time of storage in the absence of light.

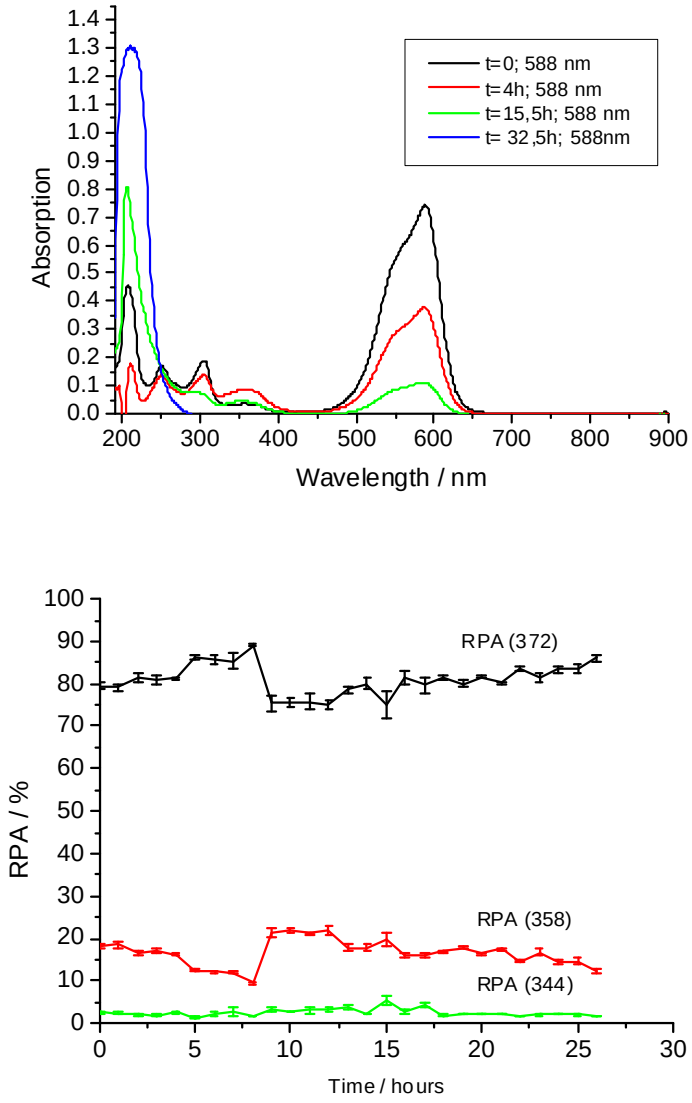


Figure 4.11 Exposure of an ethanol solution of MV (0.013 mg/ml) to xenon light (a) UV/VIS absorption spectra after different illumination time, (b) Relative Peak Area (RPA) values calculated from the MALDI mass spectra as a function of time [hours]. The RPA values describe the degradation of the dye and the production and further degradation of dye fading products.

The MALDI mass spectra gave no evidence for additional formation of degradation products (Figure 4.11b), indicating that the degradation pathways

do not include an *N*-demethylation of the dye. Degradation in ethanol was found to be complete after about 32 hours.

Because the solution evaporated during the illumination, a second experiment with volume adjustment was carried out to determine the kinetics of the degradation process. An aqueous and an ethanol solution of MV (about 0.001 mg/ml) were exposed to light during 9 hours, and absorption measurements were carried out once an hour. The concentration decrease obtained from the height of the absorption maximum of the dye was plotted against time to determine the reaction rate constant. For this purpose, the extinction coefficient of MV was experimentally determined by measuring absorption of the dye at six different concentrations and using the following equation:

$$A = \log_{10} \frac{I_o}{I} = \varepsilon \cdot d \cdot c \qquad \text{Eq. (4.4)}$$

where I_0 and I are the intensities of the incident and transmitted light, respectively, d is the path length of the absorbing solution [cm], and *c* is the concentration [mol/l]. The quantity $\log_{10} I_0 / I$ is called the absorbance *A*, while ε is known as the molar extinction coefficient [1000cm^2/mol]. Linear regression produced the following two equations from the experimental data (R^2 is the correlation coefficient):

MV in water:	$A = 23079 \cdot d \cdot c$	R^2= 0.9894	Eq. (4.5)
MV in ethanol:	$A = 20446 \cdot d \cdot c$	R^2= 0.9965	Eq. (4.6)

The decrease in absorption seen for MV corresponds to a simple exponential function, so that the process can be regarded as a first-order reaction. For this reason, only the decrease of the absorption maximum was taken into account when determining the chemical kinetics [Keuch, 2003]. Figure 4.10 shows the negative logarithms of concentration plotted as functions of time. The slopes give the rate constant *k* [s^{-1}] of the fading of MV in the two solutions [Logan, 1996]:

Aqueous solution: $-\ln(c) = 13.2026 + 2.1708 \cdot 10^{-5} \cdot t$ R^2= 0.9969 Eq. (4.7)

Ethanol solution: $-\ln(c) = 12.4546 + 4.6358 \cdot 10^{-5} \cdot t$ R^2= 0.9935 Eq. (4.8)

Accordingly, the fading reaction of MV upon exposure to xenon light was about 2 times faster in ethanol than in water.

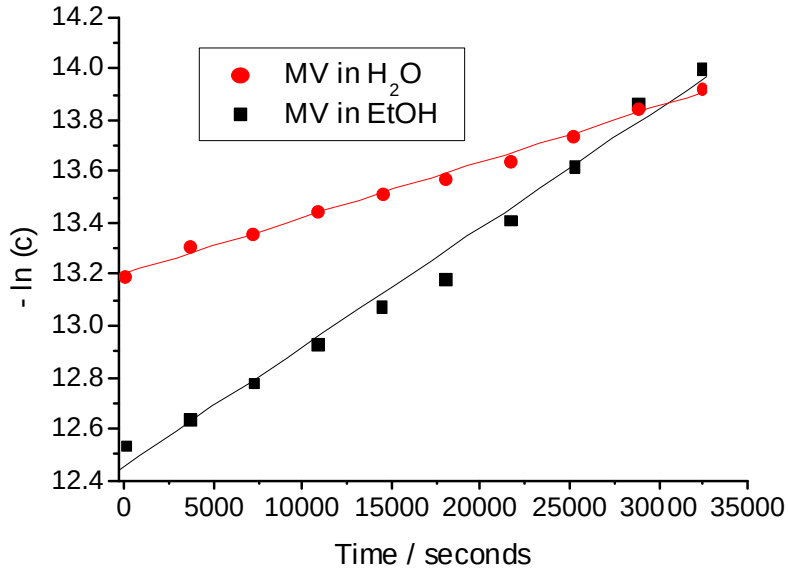


Figure 4.10 Representation of the negative logarithms of MV concentrations in water and ethanol as a function of the time [s]. The slopes as obtained from the linear regression equations yield the rate constant *k* [s^{-1}] of the pseudo first-order fading reaction. Fading is about twice faster in ethanol than in water.

The results show that significantly different pathways and kinetics compete for the fading of MV in aqueous and ethanol solution.

Signals size (peak area) in the MALDI mass spectra decreased systematically with the duration of exposure light, and no specific peaks could be identified after the complete degradation of the dye. With the aim of tracing the fading pathways of MV, other complementary methods were used.

By GC/MS analysis one can detect any volatile, but thermally stable degradation product. Therefore, aliquots of 1 µl of the aqueous and ethanol

solutions of the dye were taken after apparent complete fading, and injected in the split and splitless modes into the column. No signals in addition to those of the solvents were recorded, indicating that phenol and any other volatile products probably were not produced in significant quantities or, by the time of analysis, had already evaporated. It is conceivable, too, that some signals were hidden in the solvent signal (if the boiling points of the corresponding species were too close to those of the solvents). Alternatively, this could indicate that the degradation products are not volatile. They might then be detectable by other MS methods such as ESI-MS.

ESI-MS analysis of the pure dyes in aqueous and ethanol solution before and after the exposure to light was carried out in an effort to detect additional, elusive degradation products that might have escaped detection by other techniques. One should remember that a correlation of the presumable fragmentation patterns with the degradation products actually observed for MV may provide useful information on the reaction pathways. Unfortunately, the ESI mass spectra were complex, and an interpretation of the results rendered difficult by the many unidentified peaks seen in the background of the low-mass range. Moreover, the aqueous solutions sprayed poorly and had to be mixed with 50% by volume of ethanol to enable acquisition of the mass spectra. The spectra then obtained were characterised by smaller intensities than those found in the pure ethanol solutions.

Noticeably, both with the aqueous and with ethanol solutions of MV, the ESI mass spectra produced signals at m/z = 372 and 358. Fragmentation was characterised by signals at m/z = 357 u (Δ = 15), 358 u (Δ = 121) and m/z = 343 u (Δ = 15), 252 u (Δ = 107), 237 u (Δ = 121), respectively.

After 10 hours of illumination of MV in water, the signals at m/z= 344, 330, 316, and 302 u already mentioned were observed in the spectra. Subsequent fragmentation of m/z = 316 u produced peaks at m/z = 301 (Δ = 15), 223 (Δ = 93), 209 (Δ = 107) and 195 u (Δ = 121) which followed the same fragmentation pattern as MV in the ion trap (see figure 4.13).

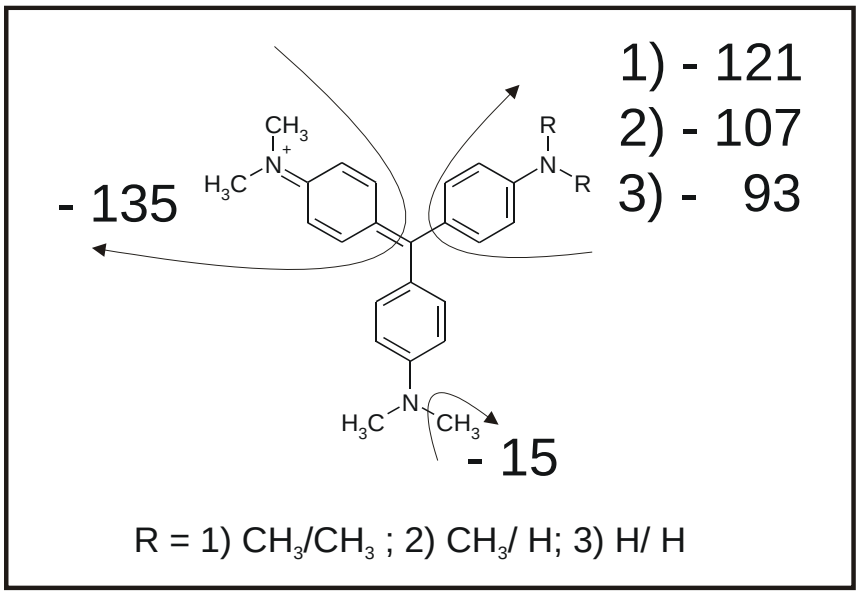


Figure 4.13 The fragmentation pattern suggested for MV, and the *N*-demethylated degradation products of MV, observed with the linear trap and identified by mass-loss analysis as follows: -15 u (corresponding to the loss of a methyl group), -93 u (loss of an aminobenzene), -107 u (loss of a methylaminobenzene), -121 u (loss of a dimethylaminobenzene) and -135 u (loss of p-methyldimethylaminobenzene moiety).

Moreover, the large peaks seen at m/z =93, 353 and 381 u are signals that can be attributed to phenylamine, the sodium salt of the pentamethylate, and the tetramethylate form of MV, respectively. An additional peak at m/z = 242 u giving rise to further fragmentation signals at m/z = 149 (Δ = 93), and 121 u (Δ = 121) may originate from the aminobenzophenone. In the negative mode, no significant signals were obtained in the mass spectra.

The degradation of MV in ethanol led to significant signals at m/z values of 269, 242, 212, 167 and 113 u in the spectra. Further fragmentation of species acting as precursors with m/z = 269 u (254 and 148 u) and m/z = 242 u (237, 149 and, 121 u) was consistent with dimethylaminobenzophenone, while the fragmentation pattern produced by the specie with m/z = 212 u (194, 167, 140) may point to dibenzylcarboxilic acid. If the ion at m/z = 167 u (with fragments at 141 and 123 u) corresponded to dibenzylmethane, this would mean that fragmentation proceeded via ring opening. It was also consistent with methylbenzenecarboxilic acid. Finally, fragmentation of the species with m/z =

112 u (at 95, 85 and 71 u) would indicate cleavage of a carbon chain with double bonds and an OH group after ring opening. In this case, the negative mode spectra gave a large signal at m/z = 135 u with a fragmentation product of 91 u, which can probably be ascribed to the corresponding ketone of the ion m/z = 269 u. A summary of the major fading products proposed is given in Figure 4.14.

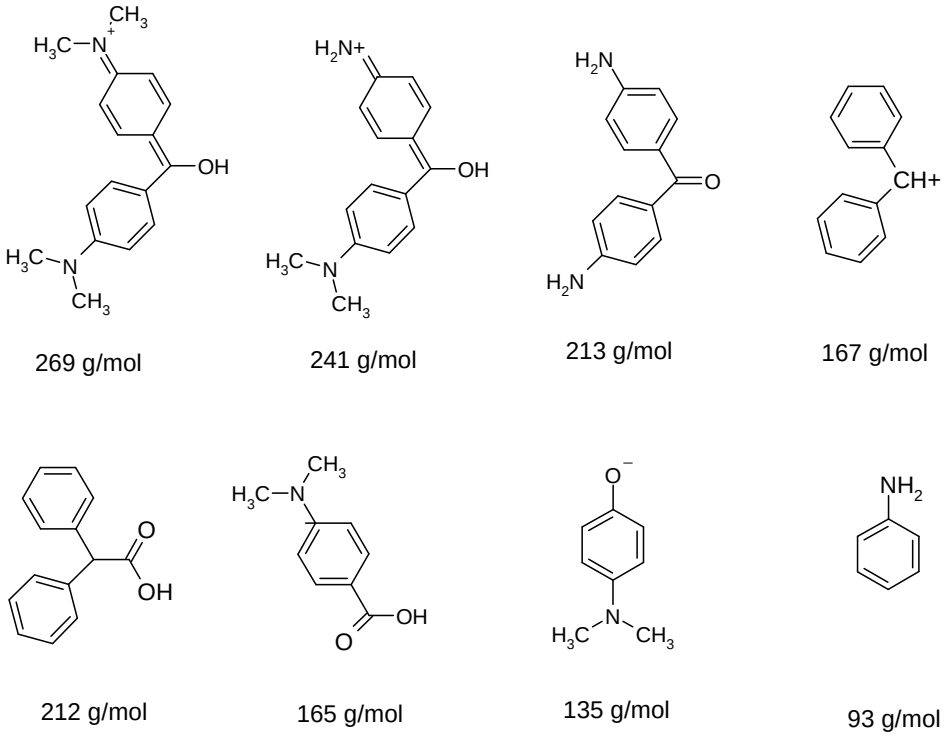


Figure 4.14 Proposed fading products of methyl violet in water or in ethanol identified by ESI-MS/MS analysis.

These preliminary ESI analyses demonstrate the great potential held by MS/MS for the analysis of dye fading pathways. Methyl violet interacts extensively with the solvents, and therefore is also used as a pH indicator for basic and acidic reactions. Brezovà et al. [2003] showed that an aqueous solution of the photo-excited triarylmethane dyes in contact with air produces singlet oxygen and ·OH radicals, while in the ethanol solution the production of $\cdot OC_2H_2$ and $\cdot O_2H$ radicals was demonstrated. For this reason, the *N*-demethylation in water probably is linked to the hydroxyl radicals attacking the

amino groups. In ethanol, the radicals generated should lead to a photo-oxidative electron/proton transfer mechanism. The role of singlet oxygen in these processes has been demonstrated while its generation in ethanol or water has not been demonstrated, although it cannot be ruled out [Brezovà et al., 2004].

Fading of ink containing MV

Light and heat

Experiments involving ink strokes gave similar results to those for the pure substances. An LDI spectrum of the BIC® blue ballpoint pen entry recorded at time t = 0 is shown in Figure 4.15. The ink contained the dyes MV (M^+ = 372.2 u) and EV (M^+ = 456.3 u). Smaller signals at m/z = 428.4 u, 358.3 u represent degradation products of the molecular ions and / or compounds contained in the ink paste composition.

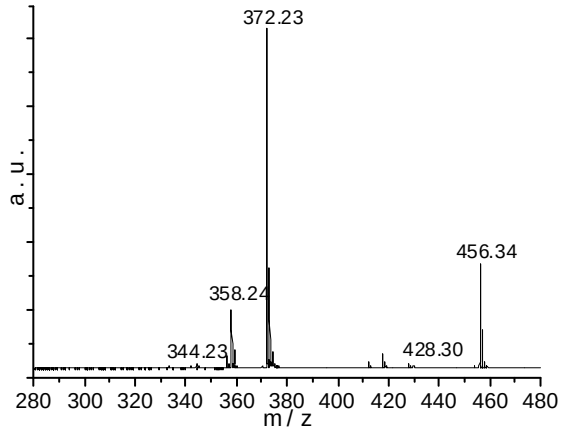


Figure 4.15 LDI-MS spectrum of a BIC® blue ballpoint pen entry on paper at time t = 0. The ink contained the dyes methyl violet (M^+ = 372.2 u) and ethyl violet (M^+ = 456.3 u). Smaller signals at m/z = 428.4 u, 358.3 u represent degradation products of the molecular ions and / or compounds contained in the ink paste composition.

No obvious difference was exhibited by the spectrum recorded after 50 hours storage in an oven at 100℃ (Fig. 4.16a). The degra dation product signals of both EV and MV, however, became very strong after 50 hours of exposure to the light of a xenon lamp (Fig. 4.16b).

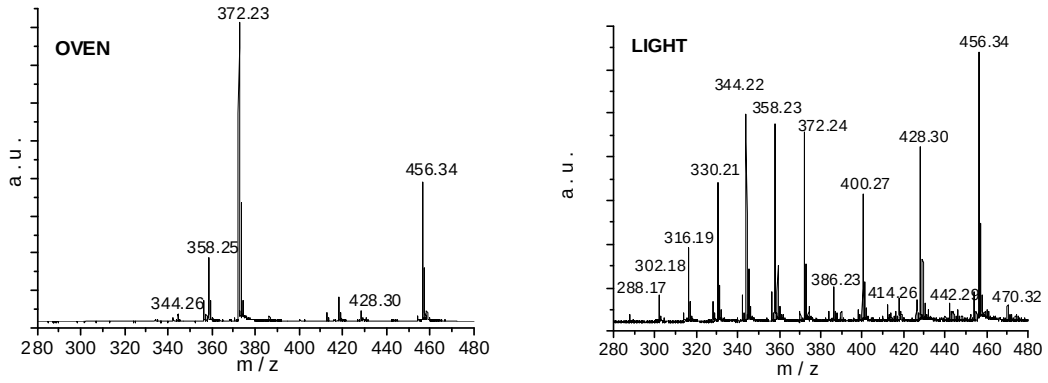


Figure 4.16 LDI-MS spectra of a BIC® blue ballpoint entries on paper recorded at time t = 50 hours: (a) spectrum of ink entries stored in an oven at 100℃, (b) spectrum of ink entries exposed to a high-pressure xenon lamp.

Aging curves confirm these observations (Fig.4.17). The aging curve of a standard which was kept in a dark box at ambient temperature was flat: no change was recorded after 50 hours. The corresponding curve for the strokes exposed to a temperature of 100℃ showed a slight d egradation of the molecular ion of EV, while the curve for strokes exposed to light of the xenon lamp exhibited a steep decrease of the signal of the molecular ion of EV: RPA_{456} attained 30% after 50 hours while RPA_{428} increased to over 20% after 10 hours already, and RPA_{400} steadily increased and reached 20% as well. No levelling off of the degradation process was observed after a time exposure of 50 hours. The xenon lamp also caused a slight increase in temperature from room temperature (22℃) to 30℃.

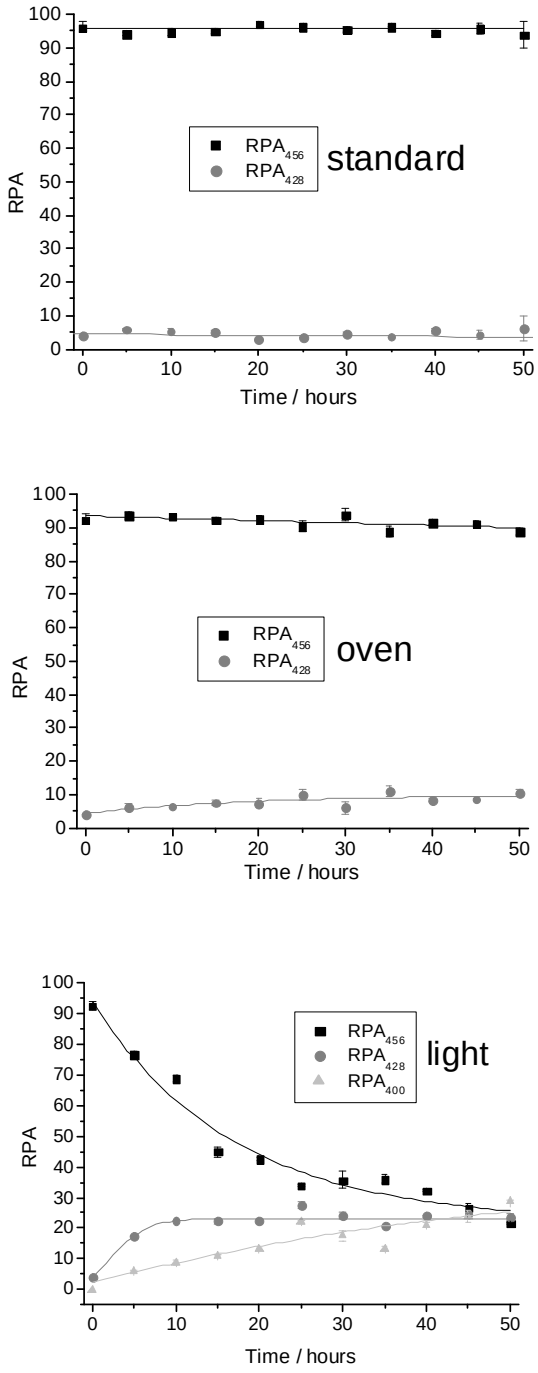


Figure 4.17 Artificial aging of EV contained in ink entries from a BIC® ballpoint pen, induced by the following treatments over a time of 50 hours: (I) storage in the dark at room temperature, (II) storage in an oven 100℃, an d (III) exposure to a high-pressure xenon lamp. The aging curves show that heat caused slight degradation of ethyl violet, while light had a strong effect already observed after a few hours exposure.

Humidity

Another interesting observation is that of enhanced degradation of the dyes MV and EV in the ink strokes upon exposure to light in humid atmospheres (Fig. 4.18). The relative humidity, $(P/P_0)\cdot 100$ where P is the water vapor pressure in air and P_0 is the equilibrium vapor pressure of water at the same temperature, was close to 100% for the humid samples, while the dry samples were those kept at ambient relative humidity (about 20 to 30%). After 9 hours exposure to light, the LDI mass spectra of dry samples led to RPA_{372} of ~ 60% and RPA_{456} of ~ 80%, while the LDI mass spectra of humid samples gave RPA_{372} of ~ 30% and RPA_{456} of ~ 60%. The pH of the paper may play a larger role under high-humidity conditions, since paper has a basic reaction with water (pH~8).

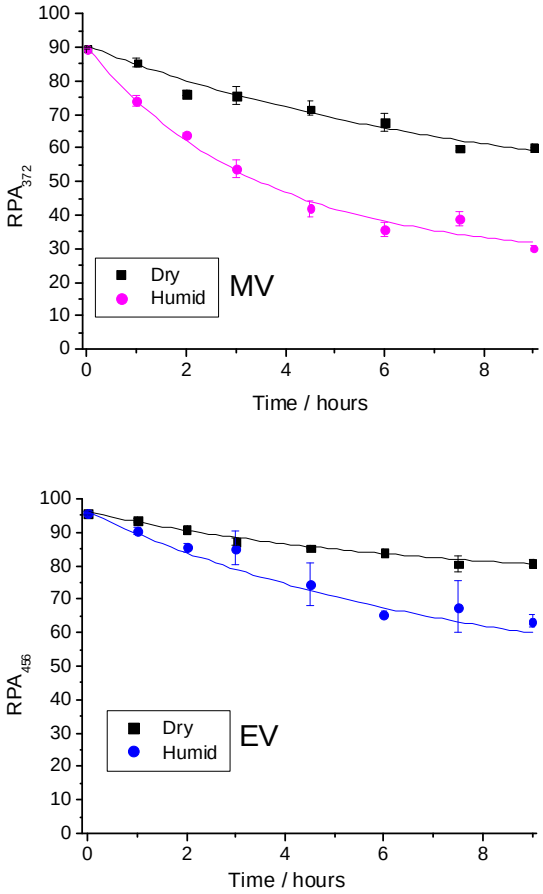


Figure 4.18 Influence of humidity on the degradation of MV and EV from ink entries of a BIC® ballpoint pen during 9 hours of exposure to the light of a high-pressure xenon lamp. The curves show that the degradation of both dyes upon exposure to light was stronger in a humid atmosphere than in a dry atmosphere.

Wavelength

The wavelength of the incident light is expected to markedly influence the degradation of the dyes in ink entries. The incident light was passed through filters so as to modify its spectrum, hence degradation could be observed under light within restricted wavelength ranges. The two dyes have their maximum absorption at 588 and 592 nm, respectively, and in addition absorb in the UV (Figure 4.19). Transmission curves of the three selected filters are shown in the same figure: the yellow filter (GG475) transmits visible light, the UV filter (SB-300-F) transmits light having wavelengths between 260 and 360 nm, and the red filter (RG5) cuts off, both the UV and the light corresponding to maximum of absorption. The experiments were run for a period of 9 hours with each filter, and for comparison without filter. The corresponding results are shown in Figure 4.20. The strongest degradation occurred when no filters was used (RPA_{372} of ~55%, RPA_{456} of ~70%). With the filter blocking, both the UV part and the region containing the absorption peak of the dye (red filter, λ>650 nm), no appreciable degradation had occurred after 9 hours of exposure to light of the xenon lamp (RPA_{372} of ~90%, RPA_{456} of ~95%). The results also show that wavelengths in the UV (λ < 360 nm) as well as around the absorption maximum of the dye (yellow filter, λ > 450 nm) produce degradation (RPA_{372} of ~75%, RPA_{456} of ~90%). These results are valid independently for each of these to regions, although with slightly different intensity. It is reasonable to assume that the fragmentation mechanisms leading to identical degradation products in each of these two wavelength regions are not the same, considering the different physicochemical behaviours of the dyes upon excitation by these wavelengths.

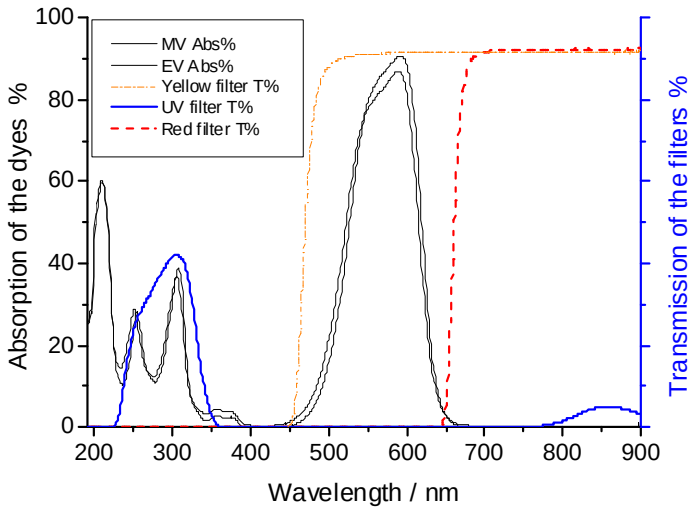


Figure 4.19 Absorption spectra of MV and EV, and spectral transmission curves of three filters: UV (SB300F), yellow (GG445) and red (RG5).

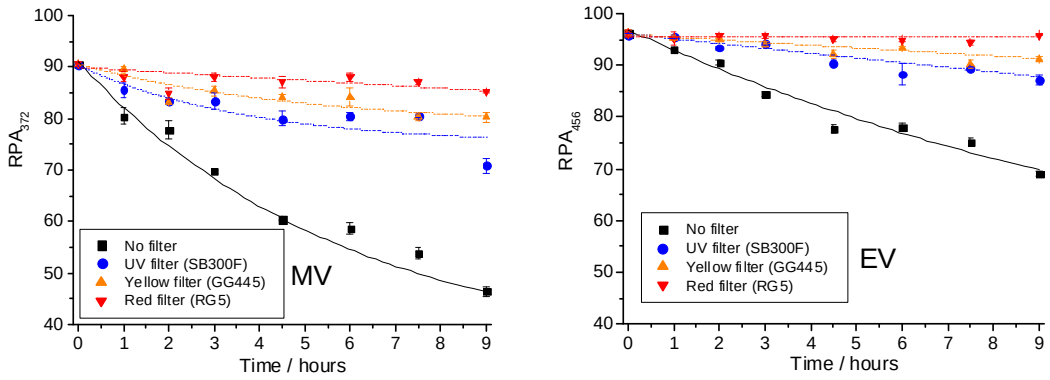


Figure 4.20 Influence of the wavelength of incident light on the degradation of MV and EV from BIC® ballpoint pen entries during 9 hours of exposure to light of a high-pressure xenon lamp. The incident light was filtered using three different filters, each leading to degradation within a restricted wavelength window.

Photon Flux

The power of the incident light per surface area was determined at a fixed distance of 17 cm with two photodiodes giving readings in volts. These values are readily converted as follows to obtain values for the irradiance in watt per cm^2 (Eq. (4.9)):

$$\frac{photodiode\ reading[V]}{amplification[V/A]*responsivity[A/W]*area[m^2]} = power\ per\ surface\ area \quad [W/m^2]$$

The values of irradiances measured without filter, with the yellow filter, and with the red filter were in the range of 10^{-6} W/m^2, while an approximate value of 10^{-7} W/m^2 was obtained in the UV range. Thus, if the incident light is filtered so as to select a particular wavelength range, it is possible to calculate the number of photons from the following equation:

$$E = \frac{h \cdot c}{\lambda} = h\nu \qquad \text{Eq. (4.10)}$$

where *E* is the photon energy in joules, *h* is the Planck's constant ($h = 6.62620 \cdot 10^{-34}$ Js), *c* is the light velocity ($c = 3 \cdot 10^{8}$ m/s) and *λ* is the wavelength of the incident light. The number of incident photons generated per unit surface area and unit time by the xenon light can be determined from the irradiance measurements and lamp spectral irradiance as a function of the wavelength (see Table 3.2). Thus, between 200 to 1000 nm, the approximate values found for the numbers of photons were at about 10^{13} s^{-1}m^{-2} in the visible range, and 10^{11} s^{-1}m^{-2} in the UV range. Hence, the number of photons generated in the UV range was about 100 times lower than that generated at the absorption maxima. On the other hand, integration of the absorption spectra of the dyes (see Fig. 4.19) showed that about 20% of the spectral absorption occurred in the UV, while 80% occurred in the visible range.

The photon flux was found to be weaker and the dyes absorbance less pronounced in the UV range. Degradation and fading nevertheless were found to be strong when provoked by UV light compared to visible light (Fig. 4.20). This can be explained by the higher photon energy of UV light leading to more reactive electronic excitations of dye molecules.

Storage conditions

These results are in agreement with the facts mentioned above that the natural aging of the ink entries is accelerated when the samples are exposed to daylight coming in through a regular room window, relative to samples not exposed to light. The BIC® ink spectra revealed no change for the reference sample between t=0 and t=349 days. In contrast, after 349 days exposure to daylight, strong signals of the degradation products of MV and EV were observed.

The aging curves in Figure 4.21 confirm these observations. No measurable change was found after one year for the ink strokes stored in the dark (RPA_{456} of ~95%, RPA_{428} of ~5%, and RPA_{400} of ~0%), whereas a significant degradation was observed, already after three days of exposure to daylight (RPA_{456} of ~35%, RPA_{428} of ~30%, and RPA_{400} of ~30%). The aging curve for ink entries exposed to daylight levelled off after 150 days. A change in colour was visible even to the naked eye: the blue colour had turned to a green-turquoise shade.

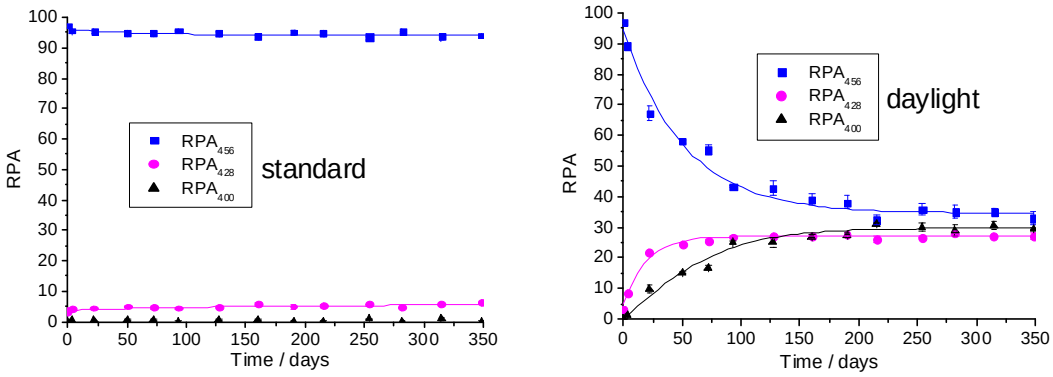


Figure 4.21 Natural aging of ethyl violet contained in ink entries from a BIC® ballpoint pen induced by the following treatments over a time of 347 days: a) standard storage in the dark, (b) exposure to daylight.

Influence of seasonal changes

Ink entries were exposed to daylight for periods of up to three years during the winter and summer seasons. Three sets of measurements were compared (winter 2001/02, summer 2002, and summer 2004). As expected, degradation of the dyes occurred within a shorter time in the spring-summer period where daily exposure to light was longer (figure 4.22). For measurements started in the summer 2002, the degradation levelled off after 100 days. Measurements started in the summer 2004 showed the strongest degradation, the levelling off occurring after 50 days. In autumn and winter, the exposure to light was shorter and direct sunshine reached the window, only after 5 p.m., therefore, the paper sheets with the strokes were irradiated mostly by scattered light during this season. Here degradation has not yet reached a limiting value after 180 days.

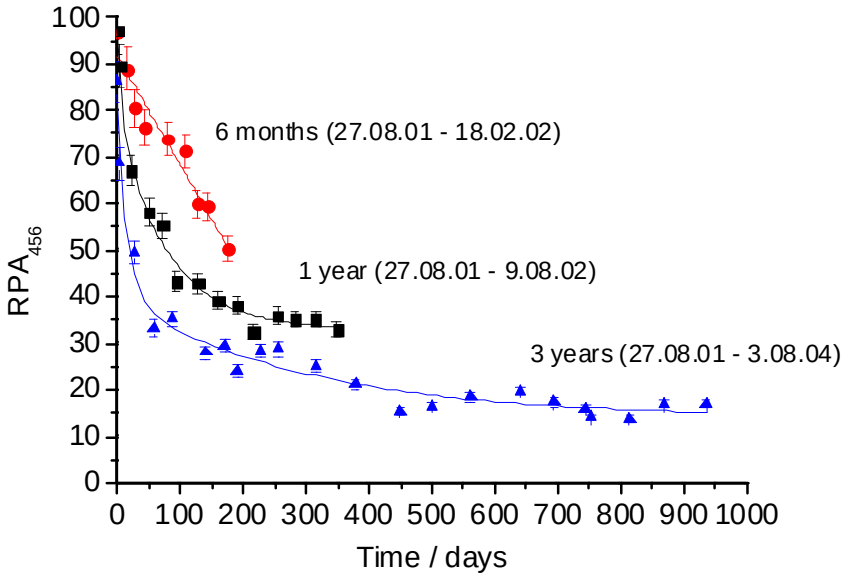


Figure 4.22 Seasonal influence: comparison of the natural aging of ethyl violet contained in ink entries from a BIC® ballpoint pen during exposure to daylight in three different periods of time. In winter of 2002 the aging was the least pronounced, while in summer of 2004 it was the strongest.

Aging Modelling

Considering how different the natural aging results were, it was clear that a simulation or modelling of natural aging (NA) behaviour by artificial aging (AA) processes would not be able to match all situations. Modelling attempts revealed large differences between the three sets of natural aging experiments conducted in this work:

- winter 2001/02: 50 hours of AA corresponds to about 600 days of NA
- summer 2002: 50 hours of AA corresponds to about 200 days of NA
- summer 2004: 50 hours of AA corresponds to about 80 days of NA

Moreover, the shapes of the curves for AA do not exactly match all types of curves for NA (see Figure 4.23). For these reasons, one cannot advocate the use of artificial aging of dyes for the purposes of dating ink from a single entry in situations where the number and energy of the incident photons reaching the ink are not known.

Daylight intensity

Photodiodes measuring visible-light and UV-light were fixed to the window for permanent measurements of the energy of the incident light impinging on the ink entries held against the window. It thus was possible to measure the power of the incident light per unit surface area as a function of time (Figure 4.24). In summer, the evening sun reaching the window caused an increase in irradiance toward the end of the day, which was not observed when clouds masked the sun. During spring and winter, the days were shorter and the amount of light measured was lower, particularly in winter. The weather was often foggy and rainy during this season, while summers were often sunny. Therefore, very different number of photons reached the window depending on season and weather.

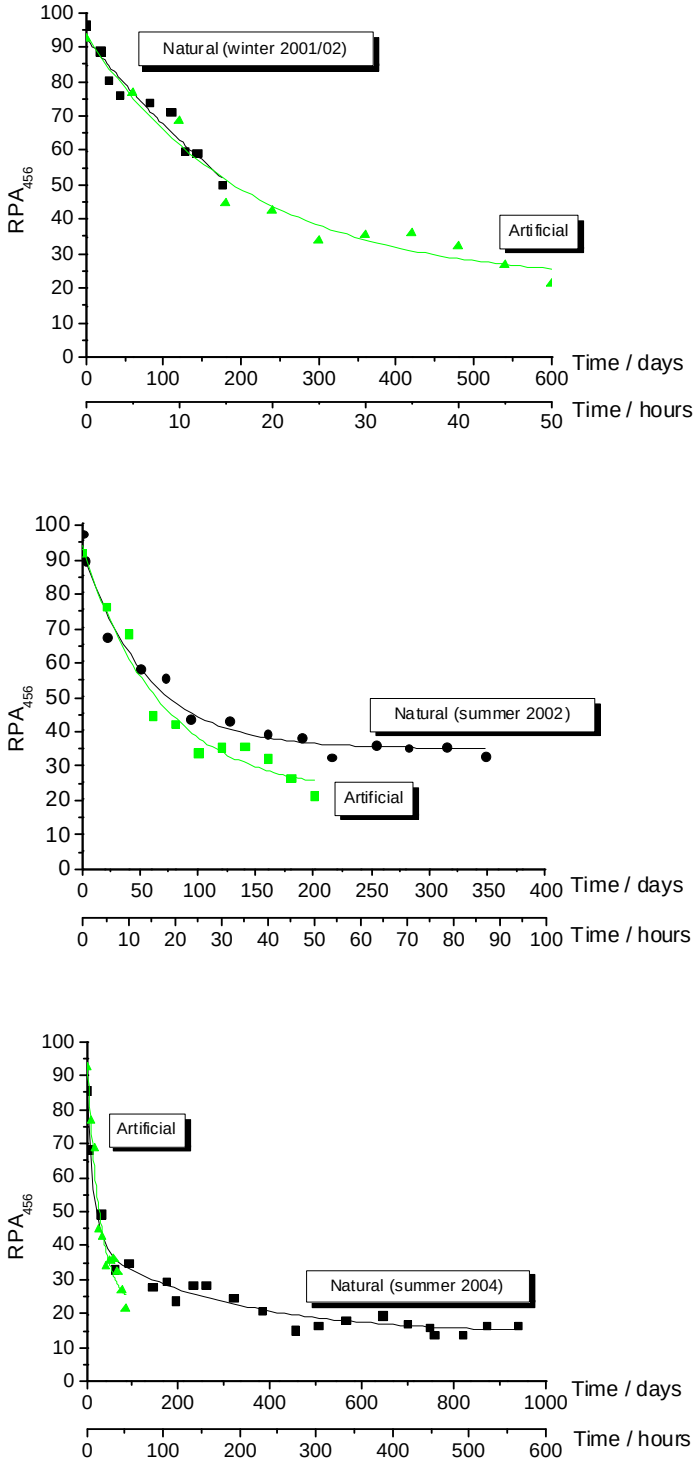


Figure 4.23 Attempts of modelling the natural aging (NA / days) with artificial aging curves (AA / hours). Very different results were obtained for the three NA experiments (winter 2001/02, summer 2002 and summer 2004) and, except for the winter of 2001/02, the modelling of NA by AA does not yield a prefect fit of the NA curves.

UV light irradiance coming through the window was about 100 times lower than that of visible light (because UV transmission was reduced by the window glass). The neon light illuminating the room and the ink entries from behind, also had an effect on aging, as it produced a response in the photo-detector facing outside the window. Moreover, a sheet of paper held against a source of light is seen to be translucent. This means that light coming through the paper probably had an additional effect on aging.

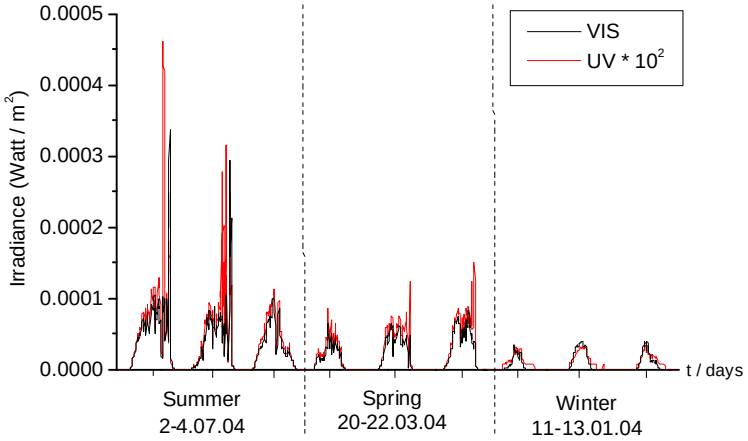


Figure 4.24 Comparison of daylight irradiance [W/m^2] coming through the window during three days in the summer, spring and, winter 2004. The irradiance of the UV light was multiplied by a factor of 100 for better visualisation. In summer, the energy increases during the evening hours as a result of direct sunshine reaching the window. On the 4th of July, clouds concealed the sun. Days are considerably shorter in spring and winter, hence the amount of light reaching the window during the day was very low.

During several summer days, temperature and humidity were recorded at the window and in the cabinet holding the ink strokes. It was observed that these parameters were rather constant in the cabinet, since almost no air flow occurred in it. At the window they strongly varied. On sunny days, the temperature increased during the day while the humidity decreased. On rainy days, the humidity reached high values. Even larger variations could be observed between summer and winter. Light, temperature and humidity were variables of influence in these natural aging experiments.

Ink composition

In this experiment, the aging behaviours of the inks from four ballpoint pens on paper were compared. The LDI mass spectra showed that the initial RPA values differ between pens (Figure 4.25). At time t=0, the RPA_{372} of MV from the ballpoint pens BIC1a, BIC1b, Herlitz and Parker were about 92%, 62%, 69% and 79% respectively. This means that the initial concentration of the dye's pentamethylated form (m/z = 358 u) differed in these four inks. No degradation upon storage in the dark could be noticed for BIC1a after three years, and for BIC1b and Parker after 2 years; however, the MV from Herlitz pen entries showed a slight degradation in the absence of light, inasmuch as the RPA value decreased to 55% after about 2 years.

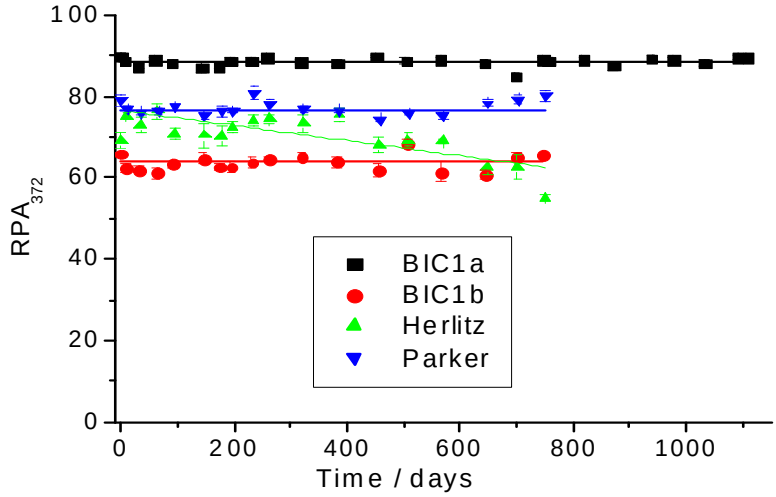


Figure 4.25 Relative peak areas (RPA_{372}) for m/z= 372 u plotted against time in days provide a basis for comparison of the fading of methyl violet from four ballpoint pens on paper during storage in the dark. Measurements have been carried out over a period of three years for the BIC1a (no fading) and during two years for the BIC 1b (no fading), Herlitz (slight fading) and Parker (no fading).

The degradation of these different inks on paper was also studied during exposure to daylight coming through a window. The fading was evident to the naked eye (Figure 4.26).

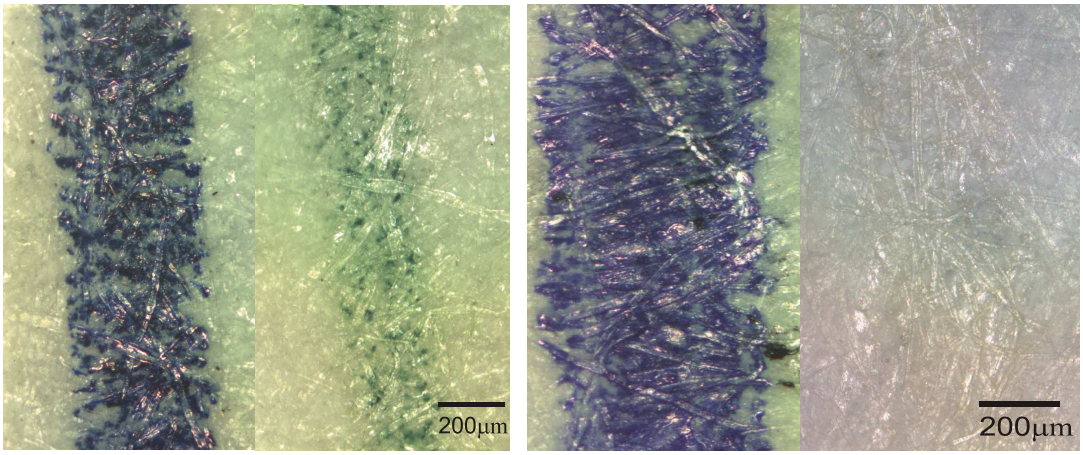


Figure 4.26 Photographies of BIC 1a and Herlitz ballpoint pen entries. Fresh ink entries (time t = 0) to the left, old ink entries (BIC 1a: t = 3 years; Herlitz: t = 2 years) to the right. The BIC 1a entries aged for three years had a greenish colour, while the Herlitz entries already had lost their colour within less than 100 days.

By LDI-MS, the *N*-demethylation reactions were detected for each of the four ballpoint pens used; however, their kinetics differed widely (Figure 4.27).

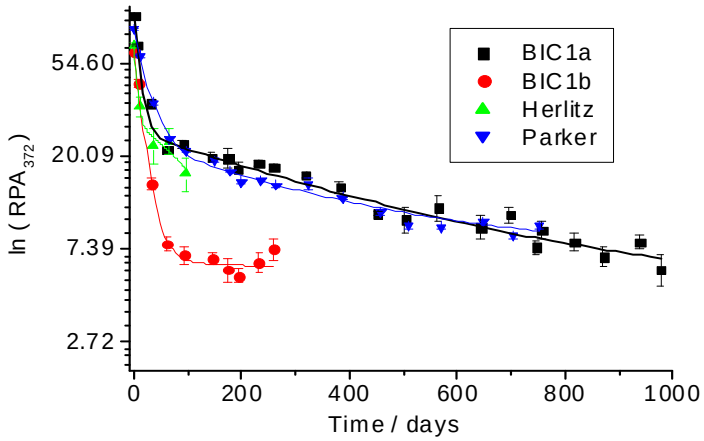


Figure 4.27 Relative peak areas (RPA_{372}) plotted as functions of time provide a basis for comparison of the fading of methyl violet from four ballpoint pens on paper during exposure to daylight. Measurements were carried out over a period of three years for BIC1a (fading complete between 977 and 1032 days), and over a period of two years for BIC 1b (fading complete between 260 and 320 days), for Herlitz (fading complete between 95 and 148), and for Parker (fading not complete after 752 days). The curves were best fitted with an exponential function.

The appearance and disappearance of degradation products in these spectra were not very distinct, unlike the situation seen for the degradation of MV in water (Figure 4.9). These products were generated simultaneously, and their RPA values did not reach levels above 30%. The time required for MV fading under identical storage conditions was found to be between 95 and 148 days for Herlitz, between 260 and 320 days for BIC1b, beyond 752 days for Parker and between 977 and 1032 days for BIC1a. The fading rates differed quite significantly between the inks. The aging curves shown were best fitted to an exponential function of the form of

$$RPA_{372} = y_0 + A \cdot e^{\left(-t/\tau\right)} \qquad \text{Eq. (4.11)}$$

where t is the time and y_0, A, τ are constants (see Table 4.3). However, the actual decays do not exactly fit the exponential function, because the degradation was not a regular process. For example, it occurred very differently on a sunny day than on a cloudy day.

	BIC1a	**BIC1b**	**Herlitz**	**Parker**
RPA_0 / %	89.55	60.92	66.23	75.84
τ / days	14.71	14.45	4.12	18.86
R^2	0.99	1.00	0.98	0.99

Table 4.3 Exponential fitting of the aging curves (figure 4.25) for the fading of MV from four different ballpoint pens during a period of 100 days. The two BIC pens had different initial RPA_{372} values but decayed with very similar slopes, while the Herlitz and Parker pens inks exhibited a stronger and weaker initial decrease, respectively.

From Eq. (4.11), the slopes of the curves at any given time are then given by:

$$RPA' = -\frac{A \cdot e^{\left(-t/\tau\right)}}{\tau} \qquad \text{Eq. (4.12)}$$

Plots of the slopes extrapolated from the exponential fittings as functions of time (Fig. 4.28) allow to observe that MV degradation was very strong during the first 10 days (-11.0 to -1.0) in the entries made with the Herlitz pen, and then

came nearly to an end after that. Degradation was least pronounced in the Parker ink entries (-2.9 to -1.7), viz. proceeding eight times slower than in the Herlitz entries over the first 10 days, while MV fading in the BIC1a entries (-4.5 to -2.3) and BIC1b entries (-3.7 to -1.9) were 1.8 and 1.5 faster than in the Parker entries over the first ten days. After 50 days, fading had slowed down considerably for all entries.

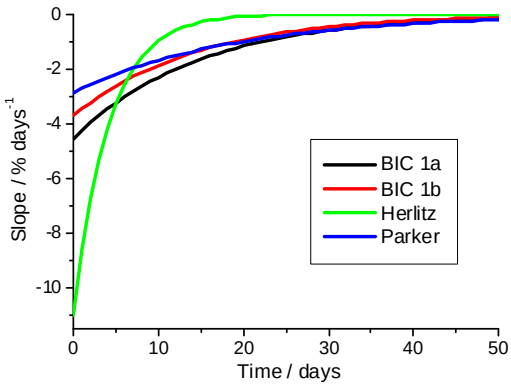


Figure 4.28 Plots of the slopes of degradation of MV [%·s^{-1}] extrapolated from the theoretical exponential fittings as functions of time [days] show that Herlitz MV degraded very strongly during the first ten days, while Parker MV degradation was least pronounced over the first few days, but was very close to MV fading in BIC1a and BIC1b inks. After 50 days, degradation had slowed down considerably for all four ballpoint pens.

The half-life period of the dye MV (symbolised $t_{1/2}$) is defined as the lapse of time necessary for the decay of half of the dye initially present. From Eq. (4.11), given that RPA = ½ RPA_0 and t = $t_{1/2}$, then:

$$t_{1/2} = 1.44 \cdot \tau \qquad \text{Eq. (4.13)}$$

The calculated half-life periods in decreasing order are then: $t_{1/2}$ (Parker) ~ 27.2 days, $t_{1/2}$ (BIC1a) ~ 21.2 days, $t_{1/2}$ (BIC1b) ~ 20.8 days and $t_{1/2}$ (Herlitz) ~ 5.9 days. The LDI mass spectra of the inks from ballpoint pen BIC1b and Herlitz contained signals of MV only, while the BIC1a spectra also contained a signal at m/z= 456 (ethyl violet), and the Parker spectra had an additional signal at m/z=470 (solvent blue 26). The two triarylmethane dyes have maximum

absorption peaks very close to that of MV, at 596 nm for ethyl violet in water and at 599nm for solvent blue 26 in ethanol. These two substances also absorbed light in the UV range. For these reasons, they competed with MV in the absorption of light and quenched the fading of MV. Their fading through the loss of CH_2 groups was also detected by LDI-MS. Moreover, blue pigments were detected by HPTLC in BIC1a, BIC1b and Parker ink entries. This could clarify why MV degradation in BIC1b ink entries was not stronger, since pigments can also act as fading quenching substances.

Ink thickness

The Parker ballpoint pen slightly smeared during writing, leaving ink blots on the ink entries that were thicker than the entries themselves. An analysis by LDI-MS after two years of exposure to daylight showed that aging was not as advanced for ink in these blots as in the rest of the entries (Figure 4.30).

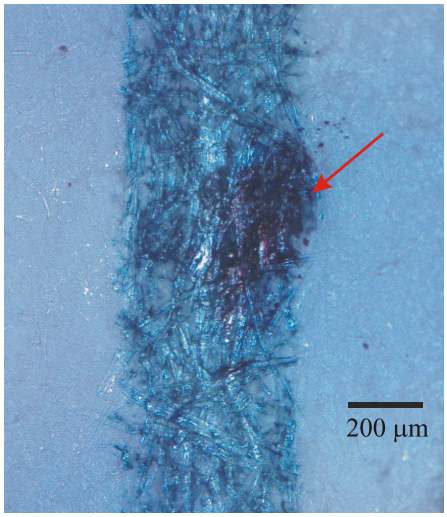


Figure 4.30 Photograph of a Parker ballpoint entry two years old: a small blot produced during writing is thicker than the rest of the entry.

The RPA_{372} in the ink entry had a value of 9%, while the same values in the two blots were 34 and 44% (Figure 4.31). Thin ink entries usually reached such values after 11 to 36 days. These results indicate that degradation depends on the local dye concentration (thickness of the ink entry on paper). Fading occurred faster in traces having a smaller local dye concentration.

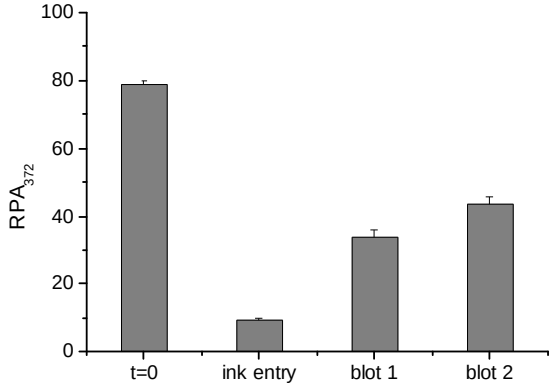


Figure 4.31 The Parker ballpoint pen during writing produced small blots that were thicker than the ink entries. Their analyses by LDI-MS after a time of about two years produced higher RPA_{372} values in the blots than in the rest of the entry, demonstrating the influence of the local amount (or concentration) of the dye on fading during exposure to light.

Testing the method on entries of known age and known composition

Ink entries of known age that had been kept in a file folder were provided by the BKA (Wiesbaden), and analysed by LDI-MS. Pieces of paper with 5 cm entries were cut, introduced into separate glass vials, and sent to our laboratory in January 2004. The entries originated from five ballpoint pens, and were created on the 27th of November 1990, the 16th of August 1999 and the 26th of November 2003, always on a different type of paper. A fresh entry was drawn for comparison on the 5th of August 2004, on a sheet of the Igepa paper. Three ballpoints pens (b5, b6, b8) contained the dye MV (m/z = 372 u), two of them (b6, b8) additionally contained the dye BB26 (m/z= 470 u). The two remaining pens contained BB7 (m/z= 478) and BB2 (m/z=484). Under a microscope, no difference in colour was observed between the old and new entries. The LDI-MS spectra recorded after 4 and 14 years differed very little. The slight decrease of the RPA_{372} values that was found does not really represent a significant degradation of the dyes, and it was not exactly the same for the three pens. The ballpoint pen b5 exhibited the largest RPA_{372} difference (12%) after 14 years (Fig. 4.32). Further confirmation is needed that the dye MV is fading very slowly in the absence of light through *N*-demethylation, by studying

samples older than 14 years; the influence of the different paper types on aging should also be tested.

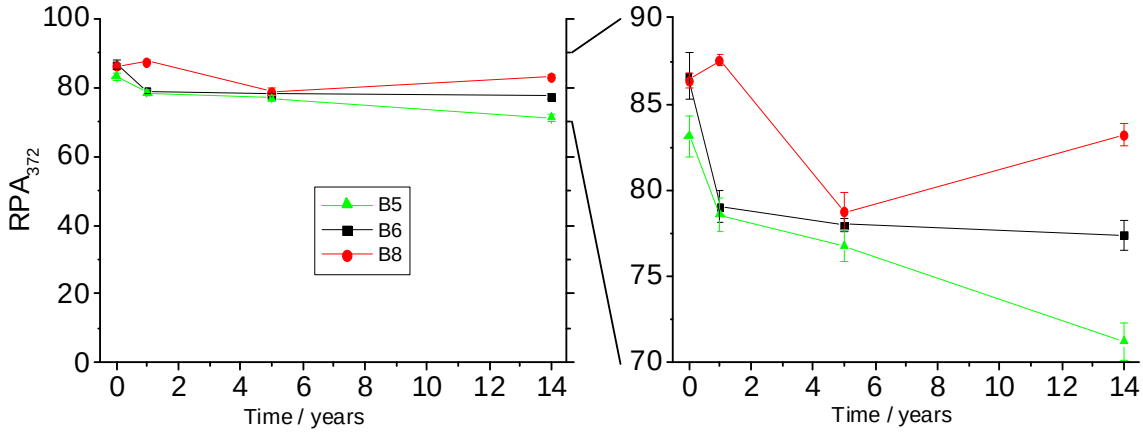


Figure 4.32 Degradation values (RPA_{372}) for the *N*-demethylation of MV in entries of three ballpoint pens provided by the BKA. A very slight fading was observed after 5 and 14 years, but remains within the limits of the relative standard deviation of 10%.

The same observations were made for two other ballpoint pen dyes, BB2 and BB7 (Figure 4.33). BB26 gave a very small signal in the LDI mass spectra, and degradation products necessary to calculate RPA values were not present in the spectra.

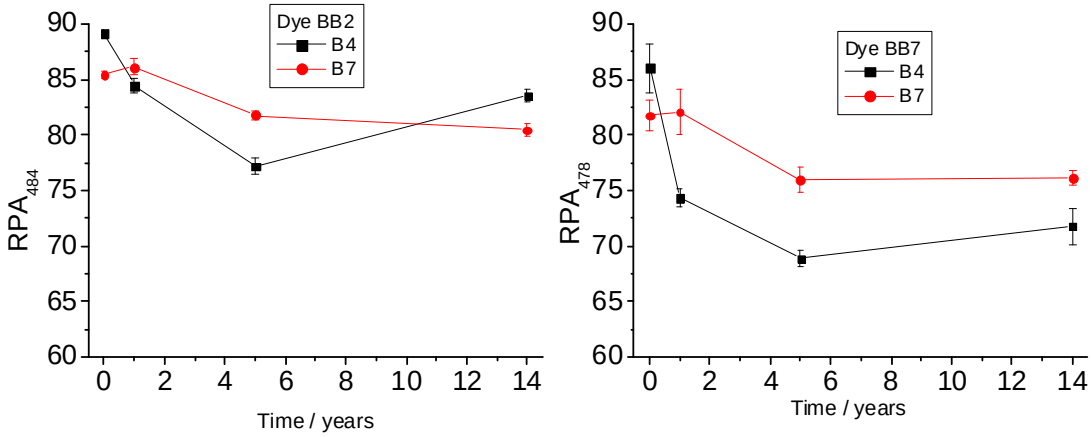


Figure 4.33 Degradation values (RPA_{484} and RPA_{478}) for the *N*-demethylation of the dyes BB2 and BB7 in entries of two ballpoint pens provided by the BKA. A very slight fading was observed after 5 and 14 years, but remains in the range of the relative standard deviation of 10%.

Spectroscopy of ink on paper

Microspectrophotometry of five ballpoint pen entries (BIC 1a, BIC 1b, Herlitz, Schneider, and Tombow) was performed directly on paper in the reflection mode (Fig.4.34). The results were affected by mean RSD between 15 and 25 %, therefore, this method can only be used for discrimination when spectra show large differences in absorption, which in the present case is true, only for the Herlitz ballpoint pen. The structure of paper and the uneven layer of ink may be reasons for this poor reproducibility. Paper has a constant absorbance of about 10% over the measured range of wavelengths. Moreover, since paper is fluorescent, measurements in the UV are not reliable.

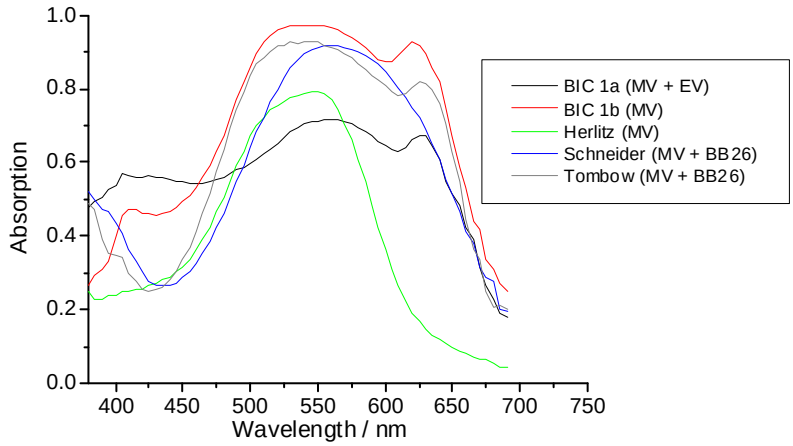


Figure 4.34 Comparison of microspectrophotometric absorption spectra of five ballpoint pens containing MV (Bic1a, Bic1b, Herlitz, Schneider and Tombow). The RSD for ten spectra can be as high as 25 %, therefore, only the spectrum obtained fo the Herlitz ballpoint pen ink was significantly different.

It was difficult to determine precise absorption maxima from these spectra, as they were very broad. After one to two years of exposure of the entries to daylight coming through a window, a decrease in absorption of 10 to 90% was observed (Fig.4.35). No absorption was detected in the Herlitz entries after one year in daylight. The spectra of samples kept in the dark, to the contrary, exhibited no significant change.

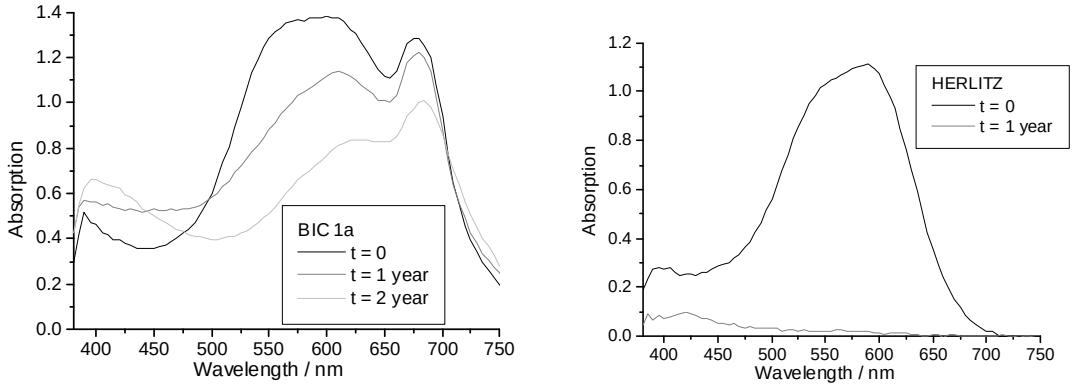


Figure 4.35 Microspectrophotometric absorption spectra of fresh and old entries from ballpoint pens BIC 1a and Herlitz. The old entries were exposed to light for 1 to 2 years, and a decrease of absorption was observed.

This method was proposed by Aginsky [1995] for dating ballpoint inks, but the low reproducibility of the results presented above shows that it is not sufficiently discriminating for a comparison of different inks on paper. The paper interference in the UV range might be removed by extracting the paper fibres covered by ink, and analysing them similarly to tissue fibres.

Line crossings (superimposed ink entries on paper)

Results obtained when analysing heterogeneous line crossings by microscopy and scanning microprobe LDI-MS are presented in this section with the aim of determining the sequence (order of writing) of superimposed ink entries. The samples investigated consisted of crossings of lines traced by a black ballpoint pen on the one hand, and lines printed by an HP Laserjet or HP Deskjet printer on the other hand. The first part of the experiment concerns the signal intensities in the LDI-MS mass spectra (Fig. 4.36).

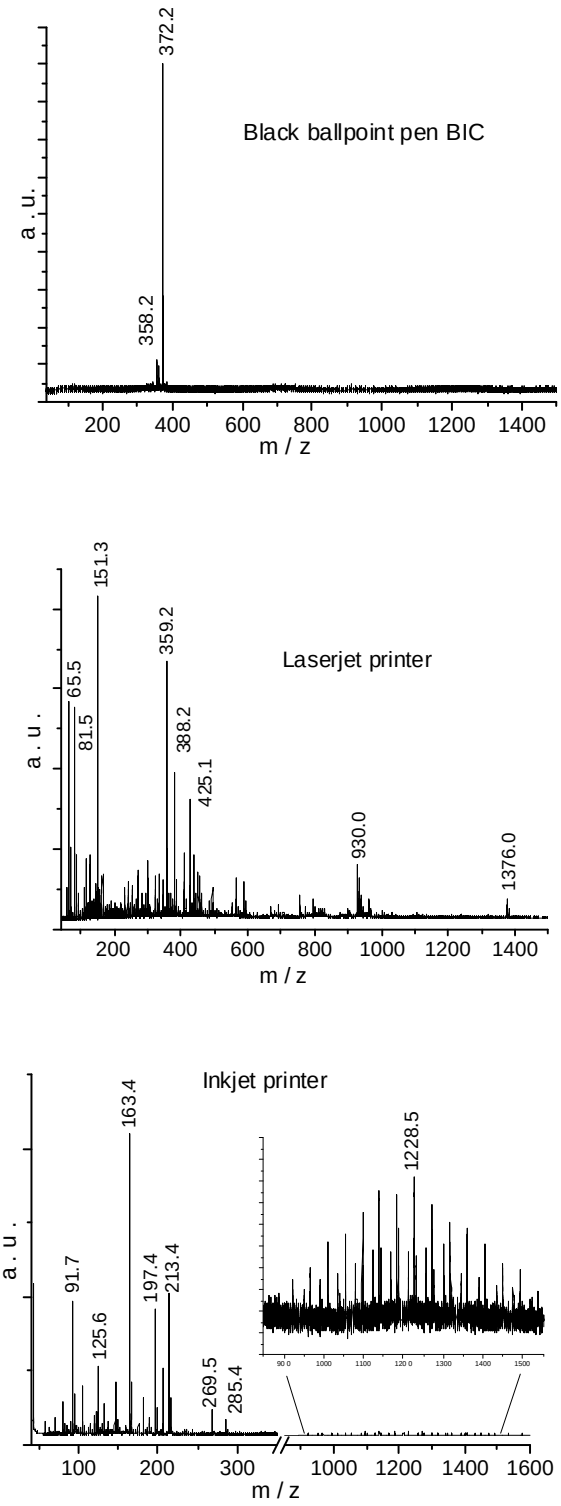


Figure 4.36 LDI mass spectra of ink entries from a black BIC ballpoint pen, an HP Laserjet printer and, an HP Deskjet printer. The ballpoint ink only contained the dye MV. Signals from the printer mass spectra remained unidentified.

Thus, a signal intensitiy factor (SIF) has been defined as follows:

$$SIF = \frac{\text{Signal Area (printer)}}{\text{Signal Area (ballpoint)}} \qquad \text{Eq. (4.14)}$$

Only one sample was evaluated for each type of crossing, therefore, the following results are single values:

- Laserjet: $SIF_{on} = 2.29$ $SIF_{under} = 0.19$
- Inkjet: $SIF_{on} = 0.37$ $SIF_{under} = 0.15$

As expected, the SIF value was larger when the printer line was above the ballpoint pen entry, particularly so in the case of the Laserjet printer.

Images of the intersection measuring about 500 µm (ballpoint pen) x 200 µm (printer) were recorded with a microscope (Fig 4.37). The crossings between the ballpoint pen line and the Laserjet printer line were easily sequenced (top/bottom), since the printer lines consist of a solidified layer of toner uniformly covering the ballpoint pen trace. The toner powder is heated for deposition and adhesion on paper. When the Laserjet line was below, shades from the ballpoint pen ink were visible on the surface of the toner layer. Inkjet ink, on the other hand, is water-based, so that the liquid solution becomes mixed with the ballpoint pen entry within the intersection. This mixing made it difficult to determine which line was above.

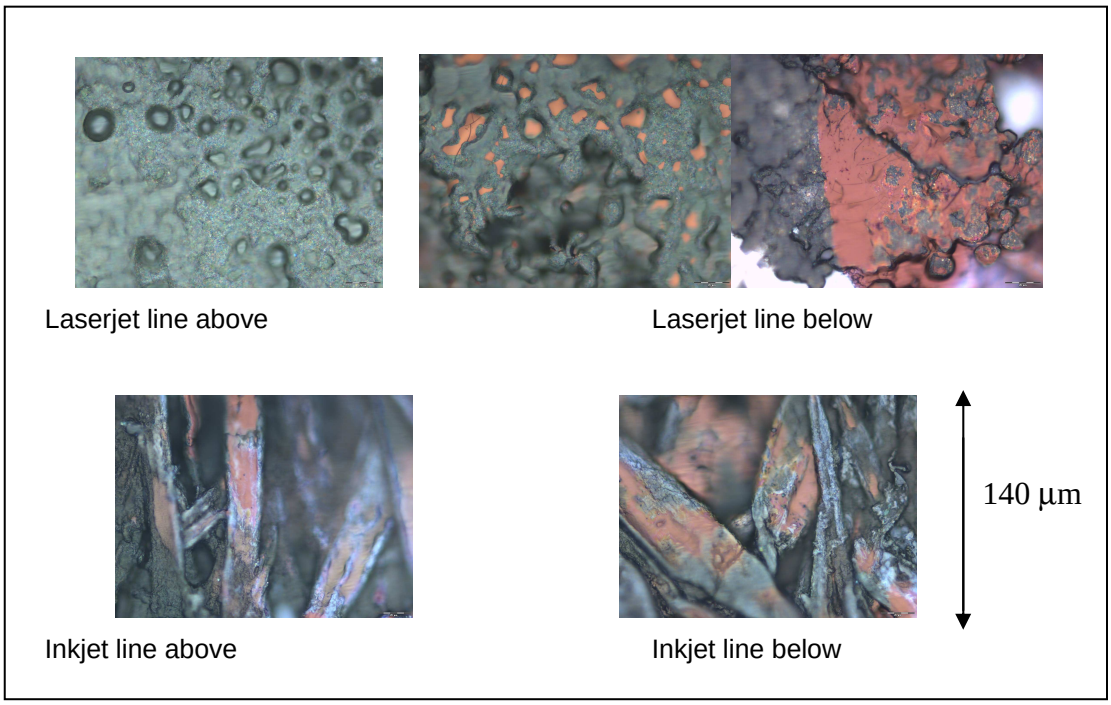


Figure 4.37 Microscopic images of heterogeneous crossing of lines created with a black ballpoint pen and with two printers. The images reveal which entry is above, in the case of Laserjet lines, but not in the case of Inkjet lines. This can be explained in terms of different surface structures of inks that have been deposited.

For scanning microprobe analysis, four signals were chosen and scanned simultaneously, including two large signals from the ballpoint pen (m/z = 372 and 358 u). As scanning was carried out by recording single spectra for each point on the intersection, the mass signals from the printer lines were much less intense than those of the ballpoint pen lines. For example, Laserjet lines did not give any signal in this mode, and the spectra of the Inkjet line had only one useful signal at m/z= 163 u. The dimensions of the scanned images were 100 x 100 µm. The results were very clear for the crossings of line from pen and Laserjet printer. With the printer line above, no signal was recorded in the spectra. To the contrary, with the printer line below, strong signals were recorded from the ballpoint pen (Fig. 4.38).

Figure 4.38 Scanning microprobe images (100 x 100 µm) from the intersection of entries from a black BIC ballpoint pen and a Laserjet printer. The two scanned signals of the BIC pen, for m/z = 372 and 358, are reported in red, while the intensity of the signal from the Laserjet printer was too low to be recorded. When the Laserjet line is above, the images are black (no signals), but when it is below, the images are red (signals of the BIC pen).

The crossings of lines created with ballpoint pen and Deskjet printer were more difficult to interpret. Usually, with the printer line above, the signals of the pen and printer were both recorded, but with the printer line below, only ballpoint ink signals were detected (Fig. 4.39). Unfortunately, some measurements made at these intersections gave opposite results (Fig. 4.40). Statistical data about the occurrence of this phenomenon were not acquired.

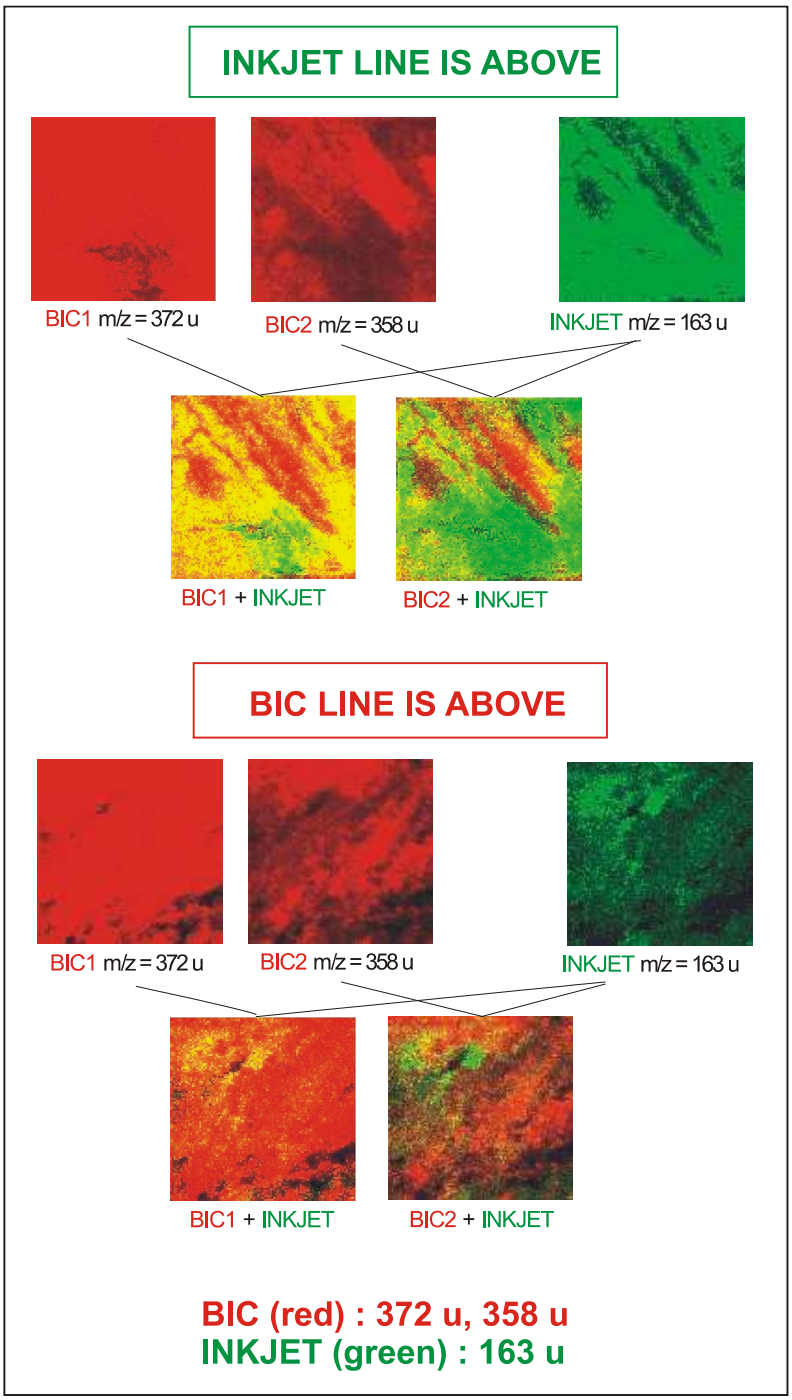


Figure 4.39 Scanning microprobe images (100 x 100 µm) from the intersection of entries from a black BIC ballpoint pen and an Inkjet printer. The two scanned signals of the BIC pen, for m/z = 372 and 358 u, are reported in red, while the signal from the Inkjet printer, for m/z = 163 u, is reported in yellow, and the superposition of the two type of signals is reported in green. With the Inkjet line was above, the images are yellow and green (signals of Inkjet and BIC pen), but with the Inkjet below, the images are red (signals of the BIC pen). The structure of the paper (fibre) is visible in the scanning images.

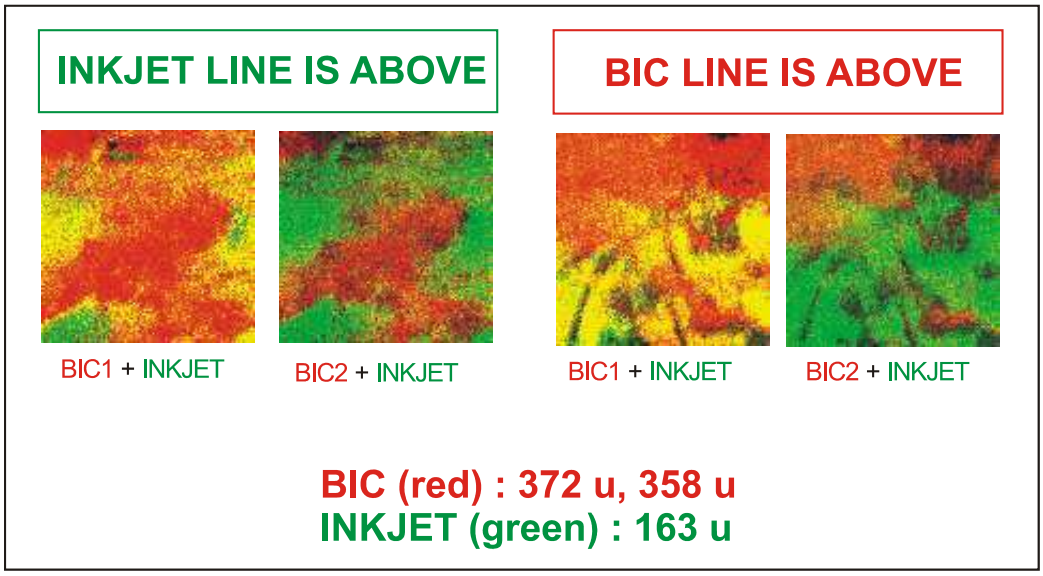


Figure 3.40 Scanning Microprobe images (100 x 100 µm) from the intersection of entries from a black ballpoint pen BIC and an inkjet printer. The two scanned signals of the BIC pen m/z = 372 and 358 u are reported in red, the signal from the inkjet printer m/z = 163 u is reported in yellow and the superposition of the two type of signals is reported in green. These are limit cases, where it is impossible to identify which signal is above from the obtained results.

The determination of the sequence of lines from a ballpoint pen and a laser printer by scanning microprobe LDI-MS was successful, because the ballpoint pen line was covered uniformly by the laser printer line, thereby suppressing the mass signals of the ballpoint pen. Crossings of lines from a ballpoint pen and an Inkjet printer were more difficult to interpret owing to a non-uniform distribution of the lines on the paper fibers, signals of both lines at times being complementary. Images should be acquired at different locations near the middle of the intersection. The printer ink mass signals were weaker than the BIC ink signals, therefore, stronger signals should be found to enhance the reproducibility and the applicability of the method.

4.2 Solvents

Qualitative and quantitative analyses of ink solvents were performed by GC/MS. The mass spectra allowed the eluted compound to be identified, which were then confirmed by comparing their mass spectra and relative retention time (RRT) to those of standard substances. The RRT is defined as the retention time of the sample, divided by that of the internal standard (IS):

$$RRT = \frac{RT(sample)}{RT(IS)} \qquad \text{Eq. (4.15)}$$

For quantitative analysis, the definition of relative peak area (RPA) has been used:

$$RPA = \frac{PA(sample)}{PA(IS)} \qquad \text{Eq. (4.16)}$$

where the area under the sample peak is divided by the corresponding area for the internal standard. Using a calibration curve it was then possible to calculate the quantities of volatile chemical species found in an ink stroke from RPA values.

In addition, the derivatisation of ballpoint reference solvents was tested with 1-naphtyl isocyanat and anthracene-9-carboxylic acid, so that less volatile derivatives of these solvents could then be analysed by MALDI-MS. The aim was to analyse dyes and solvents by the same analytical procedure. Encouraging tests in this direction had been carried out earlier with HPLC at the Bundeskriminalamt in Wiesbaden [Andermann, 2001]. Small quantities of the derivatised solvents were detected in the low range mass spectra (m/z = 100 to 500 u), however, reproducibility and intensities were unsatisfactory. Dating requirements can only be met by mass spectra delivering quantitative information on the constituents contained in the ink. MALDI-TOF-MS is not an adequate method for this purpose.

Composition

Reference substances

Two solvent standard mixtures and inks from two ballpoint pens obtained from the BKA were analysed by GC/MS. The mixtures were diluted at a ratio of 1 : 5000 in dichloromethane (DCM), and ink entries from the two pens were extracted into DCM for analysis. From the scanning mode chromatograms, qualitative results were obtained. In the mixture known as 2PH, the solvents propylene glycol (PG), hexylene glycol (HG), benzyl alcohol (BA), phenoxyethanol (P) and phenoxyethoxyethanol (PE) were found, while in the corresponding blue ink entry, only PG, HG and P were detected. In the mixture known as MBA3 and in the corresponding black ink entry, the same qualitative results were obtained, i.e. BA, P and PE were detected in both. From the information provided by the BKA, it is known that 2PH and MBA3 constituted 47.5 % and 48.9 % of the corresponding blue and black ink, respectively, prior to its application on paper. The relative fractions of the solvents in the mixtures and entries differ significantly (Table 4.4). For the blue ink, the low values for the fraction of BA (1 %) and PE (7%) in the mixture 2PH, were not found in the entries. The analysis of the ink entries also showed that the fractions of P were 28 % higher than in 2PH, and 39 % higher than in MBA3, while the fractions of the other solvents were lower. These observations indicate that the solvent P is more persistent in ink entries on paper than the other solvents.

Solvent	**2PH**	**Blue entry**	**MBA3**	**Black entry**
PG	24 %	7 %	0 %	0 %
HG	20 %	17 %	0 %	0 %
BA	1 %	0 %	31 %	4.6 %
P	48 %	76 %	60 %	98.7 %
PE	7 %	0 %	10 %	0.6 %

Table 4.4 Fractions of the solvents in the standard mixture 2PH, the corresponding blue ballpoint pen entry, the standard mixture MBA3, and the corresponding black ballpoint pen entry. Small fractions of solvents could not be detected in ink strokes on paper; the fraction of P found in the ink on paper was larger than that in the original solvent mixture, probably because P is more persistent in entries on paper.

Ballpoint pens

Ink entries of the 32 ballpoint pens available for this work were analyzed by GC-MS in the scan mode to determine the solvent mixture compositions. The following solvents were detected in the chromatograms of the 31 blue inks (Table 4.5): 94% of the ballpoint pens were found to contain phenoxyethanol, 61% contained phenoxyethoxyethanol. Benzyl alcohol (61%) and propylene glycol (54%) were other solvents often found in ballpoint pen inks, but are more volatile than those mentioned previously. Therefore, they disappear faster from the stroke, and would not be equally adequate for the dating of ink. The following solvents were also detected in some ballpoint pens: hexylene glycol (23%), ethoxyethoxyethanol and dipropylene glycol (10%), butylene glycol (6%), butoxyethanol (6%) and, in a single pen, phthalic anhydride. Typically, a specific ballpoint pen ink contained one to five of these solvents. Qualitative GC/MS results allow 15 types of ballpoint pens to be identified. Quantitative data would allow further types to be identified, but only in the case of fresh entries all being analysed at the same time t=0.

No.	PEN	P	PE	E	D	PG	BA	BG	BE	HG	PA	TOT
1a	BIC	0	0	1	1	0	0	0	0	0	0	***2***
1b	BIC	0	0	0	0	0	0	0	0	0	1	***1***
2	Caran d'Ache	1	1	0	0	0	1	0	0	0	0	***3***
3	A.T.Cross	1	1	0	0	1	0	1	0	0	0	***4***
4	Diplomat	1	0	0	0	0	1	0	0	0	0	***2***
5	Faber-Castell	1	1	0	0	1	0	0	0	1	0	***4***
6	Herlitz	1	1	0	0	1	0	0	0	1	0	***4***
7	Lamy M16	1	1	0	0	1	0	0	0	0	0	***3***
8	Lamy M21	1	1	0	0	1	1	0	0	0	0	***4***
9	Mont-Blanc	1	1	0	0	1	1	0	0	0	0	***4***
10	Parker	1	1	1	1	0	0	0	0	0	0	***4***
11	Pelikan	1	1	0	0	1	1	0	0	0	0	***4***
12	Pilot	1	0	0	0	0	1	0	0	0	0	***2***
13	Schneider Express	1	1	0	0	1	1	0	0	0	0	***4***
14	Shaeffer	1	1	0	0	1	0	1	0	0	0	***4***
15	Staedler	1	0	0	0	1	1	0	0	0	0	***3***
16	Tombow	1	0	0	0	0	1	0	0	0	0	***2***
17	Watermann	1	0	1	1	0	0	0	0	0	0	***3***
A1	Burles Industries	1	0	0	0	1	1	0	0	1	0	***4***
A2	Licher Bier	1	0	0	0	1	1	0	0	0	0	***3***
A3	Ainea AG	1	0	0	0	1	0	0	0	1	0	***3***
A5	PE SCIEX	1	1	0	0	0	1	0	0	0	0	***3***
B1	BKA blue	1	0	0	0	1	0	0	0	1	0	***3***
B2	*BKA black*	*1*	*1*	*0*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	***3***
B3	1985 PG3	1	1	0	0	1	0	0	0	1	0	***4***
B4	303/103	1	0	0	0	0	1	0	0	0	0	***2***
B5	B4466	1	1	0	0	0	1	0	0	0	0	***3***
B6	303/401 B	1	1	0	0	0	1	0	0	1	0	***4***
B7	303/110B	1	1	0	0	1	1	0	0	0	0	***4***
B8	303/701NB	1	1	0	0	0	1	0	1	0	0	***4***
B9	Ronsinco	1	1	0	0	0	1	0	0	0	0	***3***
B10	Stanis 372	1	1	0	0	1	1	0	1	0	0	***5***
%	**In the blue pens**	**94**	**61**	**10**	**10**	**55**	**61**	**6**	**6**	**23**	**1**	

Table 4.5 Solvent composition of 32 ballpoint pens determined by GC/MS (1: present, 0: absent): the solvents phenoxyethanol (P), phenoxyethoxyethanol (PE), ethoxyethoxyethanol (E), dipropylene glycol (D), propylene glycol (P), benzyl alcohol (BA), butylene glycol (BG), butoxyethanol (BE), hexylene glycol (HG) and phthalic anhydride (PA) have been identified in the chromatograms.

For further analysis, a blue Parker® ballpoint pen was chosen from the pool. Its ink contained four solvents: ethoxyethoxyethanol (E), dipropylene glycol (D), phenoxyethanol (P) and phenoxyethoxyethnaol (PE) (Fig 4.41).

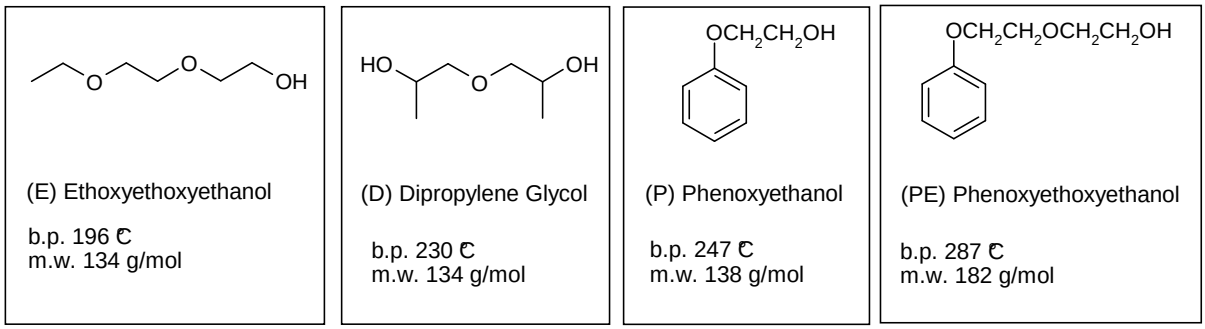


Figure 4.41 Structural formulae, molecular weights and boiling points of the four solvents contained in the Parker ballpoint pen.

Drying of pure solvents

Non porous media

The evaporation of pure solvents E (ρ = 0.999 g/ml), D (ρ = 1.023 g/ml) and P (ρ = 1.102 g/ml) was determined by weighting the mass loss from 400 µl of solvent placed into a small container having an open surface area of 9 mm^2. In this way the constant rate of drying, b, in which only evaporation plays a role, could be determined. This process can be described by a linear regression equation of the type of $m = a - b \cdot t$, where a is the mass m of solvent [µg] at time t = 0 [hours] and b is the rate of evaporation [µg/hour]:

- E: m = 444405 µg - **1713 µg/hour**$\cdot t$ R^2 = 0.9995 Eq. (4.17)
- D: m = 412501 µg - **427 µg/hour**$\cdot t$ R^2 = 0.9836 Eq. (4.18)
- P: m = 425233 µg – **157 µg/hour**$\cdot t$ R^2 = 0.9676 Eq. (4.19)

Considering that the external conditions remained constant, it can be concluded that the evaporation rates are correlated to the boiling point and saturation vapour pressure of the solvents.

Porous media

In a second group of experiments, evaporation of the pure solvents from paper was studied. The paper, because of its porous character, gave rise to larger weighting errors, but still, two stages could be distinguished in the drying process: the stage of the constant rate observed while the surface was still wet, and the stage of the falling rate, involving much slower processes (Figure 4.42). The latter took place when the quantities of solvents on the paper were very low (and errors were even higher).

Solvents were deposited on paper, and the evaporation rate was determined as a function of the time. With 20 µl solvent E, 8% of the initial weight remained after 68 hours (1770 µg). The critical point marking the end of the constant drying rate was found at about 10 hours.

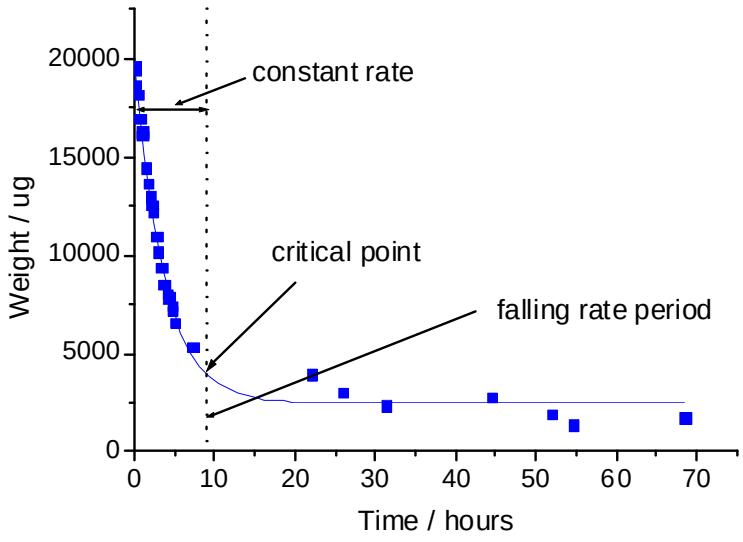


Figure 4.42 Drying curve recorded with 20 µl of solvent E on paper: the periods of constant and falling rate can be distinguished; the slope of the curve represents the drying rate [µg/hour].

Different volumes (10, 20, 40, 60, and 100 µl) of solvent E were then placed on a 10 cm^2 piece of paper in order to compare the associated evaporation rates (Fig. 4.43).

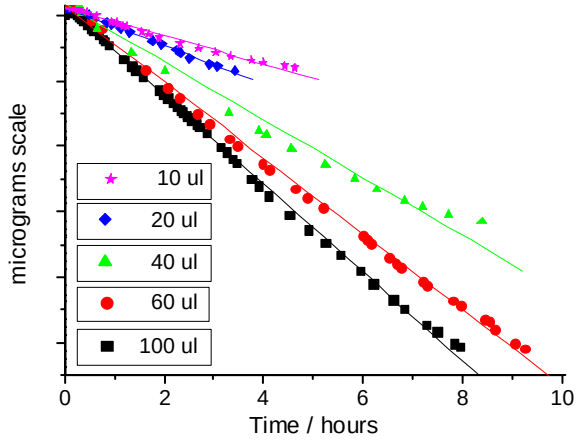


Figure 4.43 Superimposed curves for the evaporation of 10, 20, 40, 60, and 100 µl of E from paper: the loss of weight in micrograms (Δ=56372 µg) is reported as a function of the time in hours. Lower evaporation rates were found when smaller volume of solvents was initially deposited on paper.

The initial volumes (or masses) of solvent placed on the paper influenced the evaporation rate (b). The following values [µg/hour] were obtained with linear regression fits:

- 10 µl: $b = 2164 \pm 47$ µg/hour
- 20 µl: $b = 3271 \pm 42$ µg/hour
- 40 µl: $b = 4443 \pm 139$ µg/hour
- 60 µl: $b = 5817 \pm 55$ µg/hour
- 100 µl: $b = 6779 \pm 34$ µg/hour

With larger quantities of solvent applied to the paper, a larger accessible surface area will be available for evaporation (Fig. 4.44), and thus a higher evaporation rate will be observed. The visible surface areas of solvents on paper were measured as functions of the solvent volume applied. These results are indicative of the influence of variables such as solvent volume and solvent properties playing a role in the drying of solvents on porous media. Thus, the visible surface area taken up by the solvent immediately after its deposition on paper is solvent dependent. The viscosity, density, and volatility of the solvent are expected to be important here, so that for instance, P (viscosity of 22 cP) diffuses more slowly than E (with a lower viscosity of 4 cP) when a comparable

volume is applied to the paper. Solvent D, which is more viscous (75 cP) diffused even more slowly. For very small solvent volume such as those applied with the pen entries, it was not possible to distinguish a visible surface area. However, it will be seen later in this work that diffusion remains important and can be measured by GC/MS.

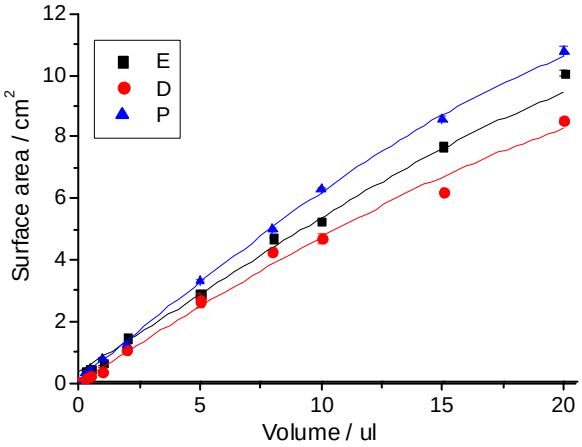


Figure 4.44 Visible surface area [cm^2] taken up by the solvents E, D and P a short time after deposition on paper, as functions of the volume deposited [µl]. The surface areas increased with the volume, but were also influenced by the viscosity, density, and volatility of the solvents.

For a comparison of evaporation rates independently of the surface area, it would be necessary to measure the solvent loss [µg] per unit surface area [cm^2] and unit time [hour]. To this end, the values obtained for evaporation of the solvents from the container (9 cm^2) were compared with the values obtained for the evaporation of 10 µl each of E, D and P from paper (where these solvents took up a surface area of 5-6 cm^2). It was seen that evaporation of E and P occurred faster on paper (Table 4.6). Two likely reasons can be given: the visible surface areas are smaller than the effective surface areas, and the superficial vapour pressure was higher in the container than on paper. The fact that evaporation of D was slower on paper than in the container is difficult to explain, but might be partly due to the fact that D does not diffuse much on paper. In addition, solvent interactions with paper, such as capillarity and adsorption, may well play a role in these processes. In fact, a solvent strongly held by fibres would have lower vapour pressure.

	Rate of evaporation [µg cm^{-2} $hour^{-1}$]	
Solvent	Container	Paper
E	190 ± 3	435 ± 47
D	47 ± 11	26 ± 2
P	17 ± 3	34 ± 1

Table 4.6 Comparison of the evaporation rates [µg cm^{-2} $hour^{-1}$] of solvents E, D and P on porous and non porous media.

The results of these experiments involving pure solvents demonstrate that the type of solvent and the initial quantity of solvent influence the evaporation rate on porous media. The structure of the substrate also influences the surface area taken up by the solvent through diffusion.

The next section is concerned with the drying of ink, i.e. of solvent mixtures. Therefore, the dynamics of the drying (diffusion and evaporation) process will depend on the properties of solvents mixtures, and altered by the interactions with others ink components.

Drying of ink

For further analysis, a blue Parker® ballpoint pen was chosen from the pool. Its ink contained four solvents: phenoxyethanol (P), phenoxyethoxyethanol (PE), ethoxyethoxyethanol (E) and dipropylene glycol (D) (see Fig. 4.45). The solvents eluted on a non polar column in the order of their boiling points and molecular weights; D produced three signals in the chromatogram, as it is composed of three isomers separated on the column. A chromatogram of the solvent blank was recorded as a reference allowing the chemical background to be subtracted. Chromatograms of the paper blank were recorded as well, but did not yield any peak. Error bars in the figures correspond to the mean standard deviation of the measurements.

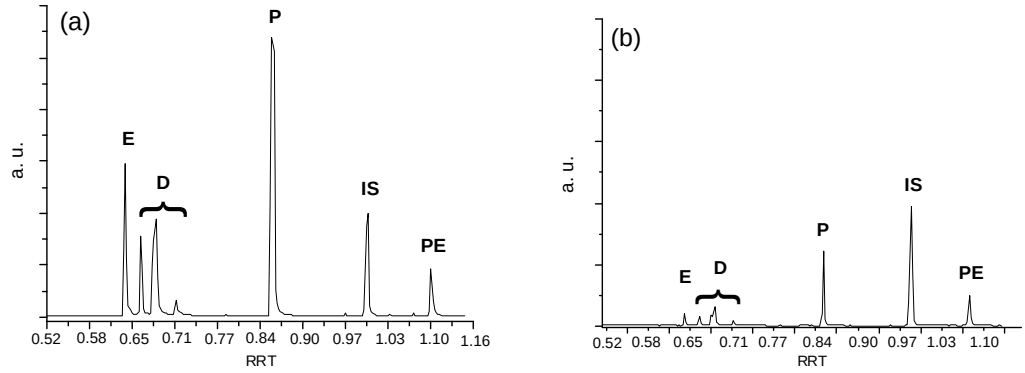


Figure 4.45 SIM Chromatograms of an extract of 1 cm Parker® ballpoint pen stroke, (a) at time t = 0, (b) at time t = 10 days after applying the ink to the paper. The x-axis represents the relative retention time (RRT) of the solvents referred to the internal standard (IS). The TIC signals of the 15 ions selected correspond to ethoxyethoxyethanol (E), dipropylene glycol (D), phenoxyethanol (P), the IS at a concentration of 0.0227 mg/ml, and phenoxyethoxyethanol (PE).

Quantitative analysis

Since no non-volatile compound belonging to the ink composition was found in the chromatograms (i.e. no peak stable in intensity over time), it was necessary to introduce an internal standard (IS) to perform quantification. The relative peak area (RPA) for any given substance S_i was calculated as follows in order to minimize the error:

$$RPA = \frac{\text{Peak Area of } S_i}{\text{Peak Area of IS}} \qquad \text{Eq. (4.20)}$$

The RPA is a measure of the quantity of solvent in the stroke relative to that of the internal standard. Using this definition, a set of linear calibration curves was obtained by measuring six different points in the concentration range from 0.005 to 0.1 µg / cm for E, D and P as reference substances. The following expressions describe these curves:

- $RPA_E = c_E \cdot 6.9609$ $\quad R^2 = 0.9401$ Eq. (4.21)
- $RPA_D = c_D \cdot 5.6275$ $\quad R^2 = 0.9643$ Eq. (4.22)
- $RPA_P = c_P \cdot 10.644$ $\quad R^2 = 0.9933$ Eq. (4.23)

Where, c_i is the concentration [µg/cm] of the target substance i, and R^2 is the regression coefficient of the fitting.

Twelve ballpoint pens containing at least one of the solvents E, D and/or P were selected for a comparative quantitative analysis of their ink entries at time t = 0. The results (see Table 4.7) show that initial quantities of these solvents may significantly diverge between ballpoint pens. For instance, the ballpoint pen B4 had about 5% of the P content of B8. It is an important conclusion resulting from this observation that the initial quantitative composition of an ink should be known for a correct determination of the age of ink strokes from solvent loss measurements on ballpoint pen entries.

No	**Pen**	**Initial amounts of solvent [µg]**		
		E	**D**	**P**
B8	303/701NB	0	0	0.66
B6	303/401B	0	0	0.57
B9	Ronsinco	0	0	0.53
B1	BKA blue	0	0	0.42
B4	303/110B	0	0	0.41
B5	B4466	0	0	0.38
10	Parker	0.21	0.33	0.30
9	Mont-Blanc	0	0	0.22
6	Herlitz	0	0	0.22
17	Watermann	0.18	0.25	0.20
B4	303/103	0	0	0.03
1a	BIC	0.29	0.83	0

Table 4.7 Initial quantities [µg] of the solvents ethoxyethoxyethanol (E), dipropylene glycol (G), phenoxyethanol (P) in 12 blue ballpoints ink entries. The quantities vary significantly between pens even at time t=0, that is, immediately after applying the ink to the paper.

Weight of a stroke

Quantitative analysis of the ink in the Parker ballpoint cartridge gave a total weight percentage of solvents of approximately 53% (excluding PE, since it was not available as a standard for quantification), which was distributed as follows: 24 ± 8 % for E, 11 ± 1% for D and 18 ± 2% for P.

As explained before, the mass of 1 cm ballpoint pen entry was determined by weighting, both the loss of ink in the ballpoint pen (c_1) and the gain in ink on a piece of paper after writing (c_2). Six replicate measurements gave c_1= 6.63 ± 0.49 µg/cm and c_2= 4.63 ± 0.71 µg/cm, respectively. The higher error of c_2 may have to do with the facts that paper is a porous material and solvent evaporation occurs even during the measurements. In comparison, the ballpoint pen cartridge is a closed environment, and evaporation is minimised. It must be expected, then, that c_1 provides a more precise estimate of the ink mass in a stroke. Another source of error is the uneven application of the strokes, particularly when ink accumulates on the ball and is deposited as a thick mass at the beginning or the end of a stroke. From the above results, the following initial concentration (c_i) of E, D and P in 1 cm stroke can be extrapolated from the cartridge weight loss (c_1): c_i (E) = 1.59 ± 0.69 µg/cm, c_i (D) = 0.73 ± 0.11 µg/cm and c_i (P) = 1.20 ± 0.23 µg/cm.

Aging

For better visualisation, aging curves of ink entries in terms of solvent loss were obtained for each solvent by plotting the RPA values as functions of the square root of time in hours (which is the usual way of display for drying curves, Fig. 4.46a). The RPA values decreased very quickly over the first three hours. After two weeks, the rates of drying considerably slowed down. The curves were best fitted with an expression proposed by Lociciro et al. [Lociciro et al., 2005]:

$$RPA = p_1 + p_2 \cdot e^{-\left(\frac{t}{p_3}\right)^{0.5}} + p_4 \cdot e^{-\left(\frac{t}{p_5}\right)^{0.5}} \qquad \text{Eq. (4.24)}$$

A double logarithmic scale yields a better representation of the decrease of the solvent content in the ink entries as a function of time (Fig. 4.46b). A double logarithmic curve fit produced regression factors R^2 between 0.9346 and 0.9965. The RPA values for E decreased very quickly at the beginning, and became nearly constant after about 10 days. On the other hand, disappearance of the solvents D and P could still be measured after 562 days. The RPA values found for PE were lower than those for other solvents, and decreased more slowly with time. From these results, it can be concluded that the phase of drying at a constant rate that had been discussed above ends rather quickly, and cannot be distinguished in measurements involving such small quantities. The first derivative of the drying curves will then reflect the phases of drying with falling rates. This interpretation can be applied to Eq. (4.21) where the first exponential term represents the stage with the 1st falling rate (diffusion to the surface), and the second exponential term describes the stage of the 2nd falling rate (when physical adsorption has occurred).

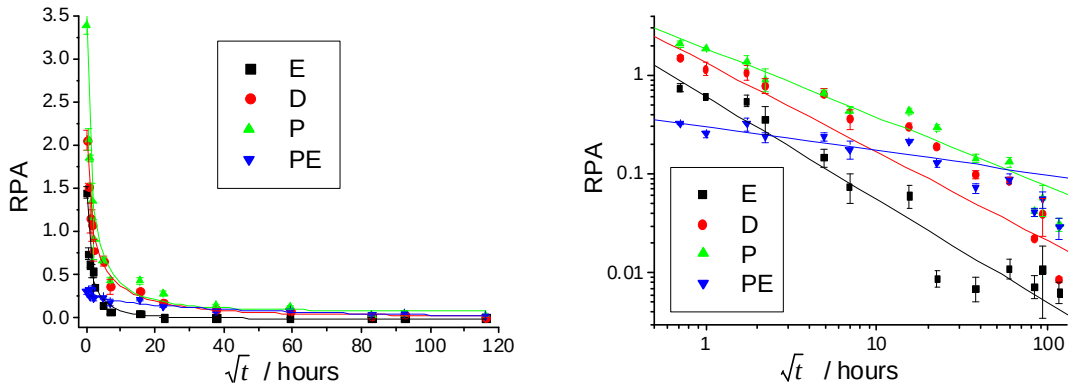


Figure 4.46 Drying / aging curves of ink strokes in term of solvent loss for ethoxyethoxyethanol (E), dipropylene glycol (D), phenoxyethanol (P) and phenoxyethoxyethanol from Parker® ballpoint pen entries: (a) RPA (Relative Peak Area of solvent to the IS) as a function of the square root of the time in hours, (b) RPA as a function of time on a double logarithmic scale. These curves can be interpreted by assuming that the solvents disappear from the stroke via the combined processes of evaporation and diffusion.

The concentrations of solvent in the stroke at time t can be calculated directly from the RPA values (see Fig. 4.47). These concentrations decreased very quickly, reaching levels below 0.1 µg/cm after ten days and levels in the ng/cm range after one year. For time t = 0, the following values were determined: $c_{t=0}$ (E) = 0.21 ± 0.01 µg/cm, $c_{t=0}$ (D) = 0.33 ± 0.02 µg/cm, $c_{t=0}$ (P) = 0.30 ± 0.03 µg/cm. By reference to the extrapolated values of solvent loss from the cartridge (c_i) reported above (which represent the amounts of solvent transferred to paper from the pen), it is possible to conclude that 87 ± 5 % E, 55 ± 15 % D and 75% ± 8 % P disappeared from the stroke within the few seconds after drawing a ballpoint line. This corresponds to a combined loss of solvent of 76 ± 9 % (if we disregard PE, which has not been quantified). The individual values indicate that a solvent with a higher boiling point (P) disappeared more quickly from the stroke than another one with a lower boiling point (D). This can only be explained by the competition between evaporation, diffusion and adsorption. Also, the friction between the ballpoint and the paper may slightly heat the ink during its application, which represents an initial energy input favouring initial evaporation.

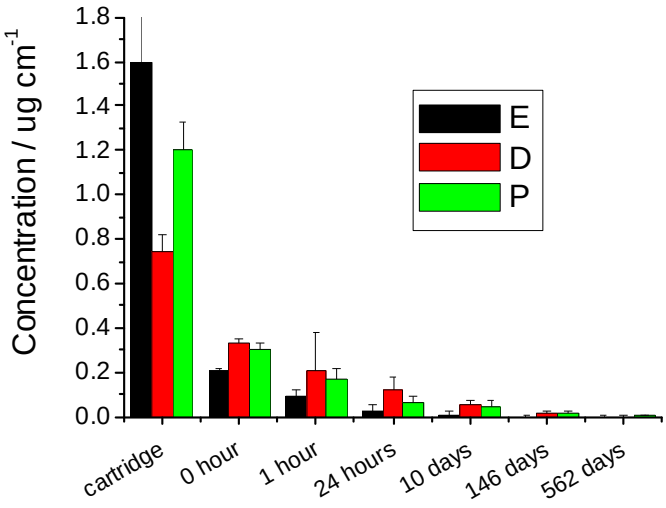


Figure 4.47 Masses per unit stroke length of the solvents ethoxyethoxyethanol (E), dipropylene glycol (D), phenoxyethanol (P) and phenoxyethoxyethanol from a Parker® ballpoint pen, determined from the cartridge weight loss and from GC/MS measurements of residual concentration at different times after application on paper.

The mean relative standard deviation (RSD) of three measurements was typically between 5 and 30 % (there was a single case involving ethoxyethoxyethanol where RSD was as high as 68%). As expected, the RSD increased as the residual ink mass on paper decreased. GC/MS measurements from fresh ink entries immediately after their application on paper (t=0) were carried out over a few weeks and had a mean RSD below 10%. After a few months, the RSD had increased up to 30 %.

Ink composition

The drying of the two solvents E and D were compared in ink entries made by two different ballpoint pens. The ink of the BIC1a ballpoint pen contained only these two solvents, while the Parker pen contained P and PE as well. Strangely, the drying rate of E is slightly higher in the BIC1a strokes, the drying of D is slightly faster in the Parker entries. Also, E was no longer detected in the BIC1a ink entries after 48 days, while small quantities of E were still found in the Parker ink entries after more than one year (Fig. 4.48). This could mean, either that solvent dynamics were different in view of the differences in ink composition, or that small variations in external factors (temperature, air flow, adjacent material) had a significant influence on the drying.

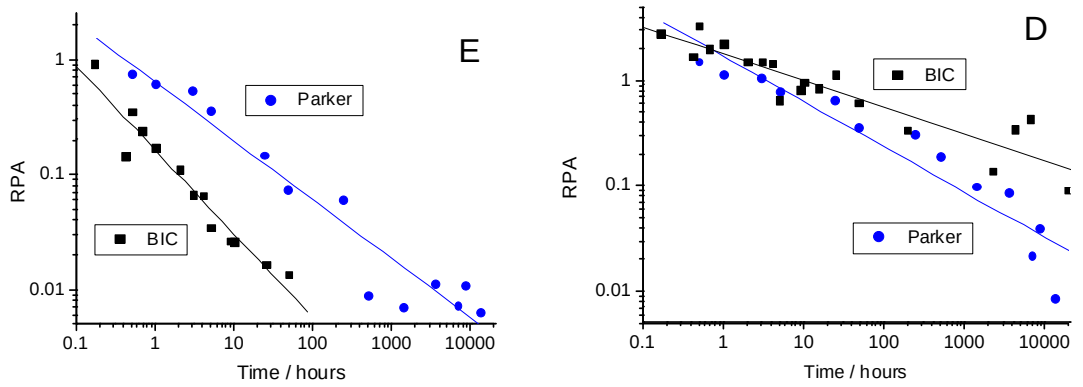


Figure 4.48 Comparison of the drying of E and P on paper from the ink entries of two ballpoint pens, BIC1a and Parker by GC/MS. For improved visualisation, logarithmic scales were used for the RPA (Relative Peak Area of solvent) and for the time scale. The drying rates differ significantly between the two inks.

Modelling of aging

It has been proposed in the literature to date inks from one single entry by subjecting the entry to artificial aging in an oven at a temperature of 60 to 80℃ [Aginsky, 1996]. To test this suggestion, ink entries made with the Parker ballpoint pen were stored for 17 days in an oven at 60℃ while performing quantitative GC/MS measurements at different times (fig. 4.49).

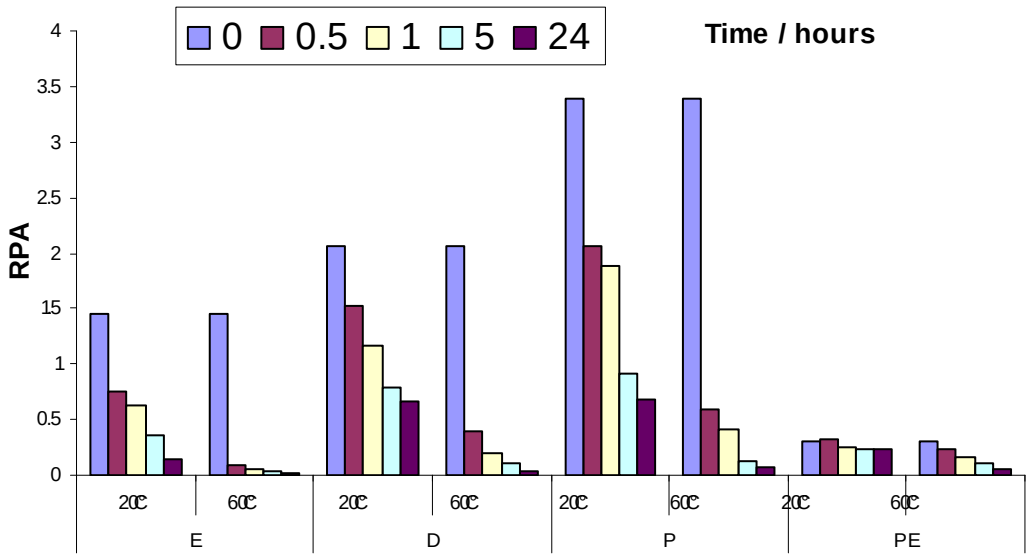


Figure 4.49 Solvent losses from ink entries by GC/MS: comparison of Parker ink entries stored at 20℃ and at 60℃. Measurements were perfo rmed at times of 0, 0.5, 1, 5 and 24 hours after application of the stroke. All four solvents decrease more quickly at higher temperature.

An attempt was made to simulate or model natural aging (20℃) by artificial aging (60℃) (Fig. 4.50). The following results wer e obtained for the four solvents:

- E: 18 hours at 60℃ corresponds to about 300 days at 20℃
- D: 18 hours at 60℃ corresponds to about 300 days at 20℃
- P: 18 hours at 60℃ corresponds to about 400 days at 20℃
- PE: 18 hours at 60℃ corresponds to about 200 day s at 20℃

The results are unexpected, inasmuch as accelerated aging was the strongest for a solvent with a rather high boiling point (P), and differed significantly between solvents. The artificial aging curves yield to rather good fits of the natural aging curves, but it is imperative to verify that the modelling does not change between ballpoint pens having different ink composition. It is possible, moreover, that solvent molecules become desorbed at the higher temperature, while at normal temperature they would be physically attached to the paper.

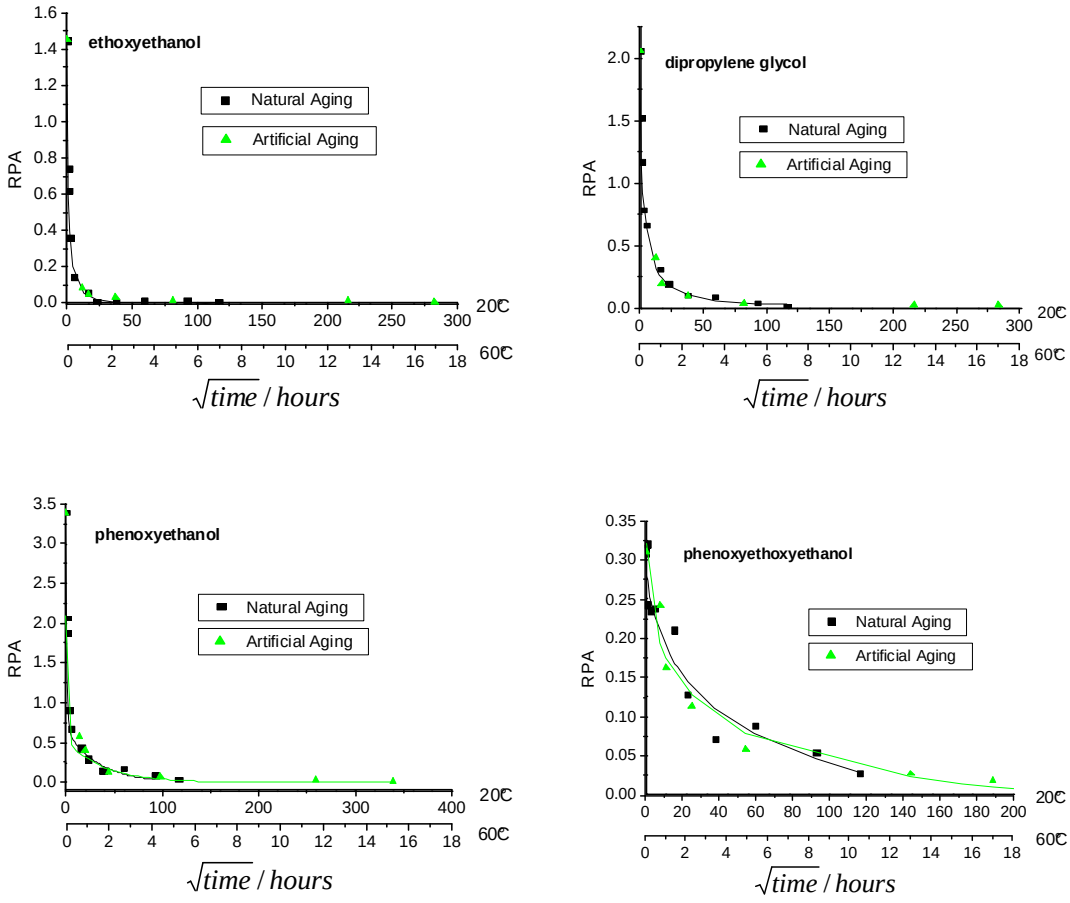


Figure 4.50 Attempt to model the natural aging (20℃) by artifi cial aging (60℃) curves. RPA values are reported as functions of the square root of time. Different results were obtained for the four solvents E, D, P and PE. Surprisingly, it is the solvent P with a high boiling point, whose aging is most strongly accelerated by an increase of the temperature.

Diffusion and migration

For a quantitative determination of solvent diffusion, the concentrations of solvents were measured at distances of 2, 4, 6 and 8 mm from the ink stroke, at times ranging from t = 0 to t = 12 days (fig. 4.51). Solvents E and P diffused quickly in the paper (low viscosity), while D diffused more slowly (high viscosity). No diffusion of PE was detected in the area next to the stroke.

The points in the plots of Figure 4.51 were joined by a Gaussian curve fitting to obtain diffusion curves (the areas under the curves represent the amount of solvent per unit length of stroke). These curves can be integrated to estimate the mass of solvent disappearing from the paper through evaporation and by eventual migration out of the paper (see below). The decrease in area which occurred as a function of time approximately represents the loss of solvents from the paper (diffusion to areas further than 8 mm from the ink entry was neglected, as it has not been measured, but it is presumably quite insignificant when considering the tendencies in the plots). Table 4.8 shows the percentages of solvents lost from the paper (by evaporation) and from the stroke (by evaporation and diffusion). The values were extrapolated from data obtained by integrating the diffusion curves (in approximation of sole evaporation) and by quantitative GC/MS analysis (residual solvent in the ink entry). Solvents E and P diffuse well in paper, hence the evaporation surface area is larger and they disappear quickly within the few seconds after application. Solvent D diffuses more slowly, and therefore, evaporation is also slower.

% in 1cm ink entry	E	D	P
Cartridge	100%	100%	100%
Loss from paper (t=22s)	56%	11%	44%
Loss from stroke (t=22s)	87%	56%	75%

Table 4.8 Loss of the solvents ethoxyethoxyethanol (E), dipropylene glycol (D), phenoxyethanol (P) from 1 cm stroke determined a few seconds after application of the stroke to the paper (in percent). The loss of solvent from the paper is assumed to be mainly due to evaporation, while the loss from the stroke is due to competitive evaporation and diffusion. Solvents E and P diffuse well in paper, hence the evaporation surface area is larger and they disappear quickly (within a few seconds after application). Solvent D diffuses more slowly, and therefore, evaporation is also slower.

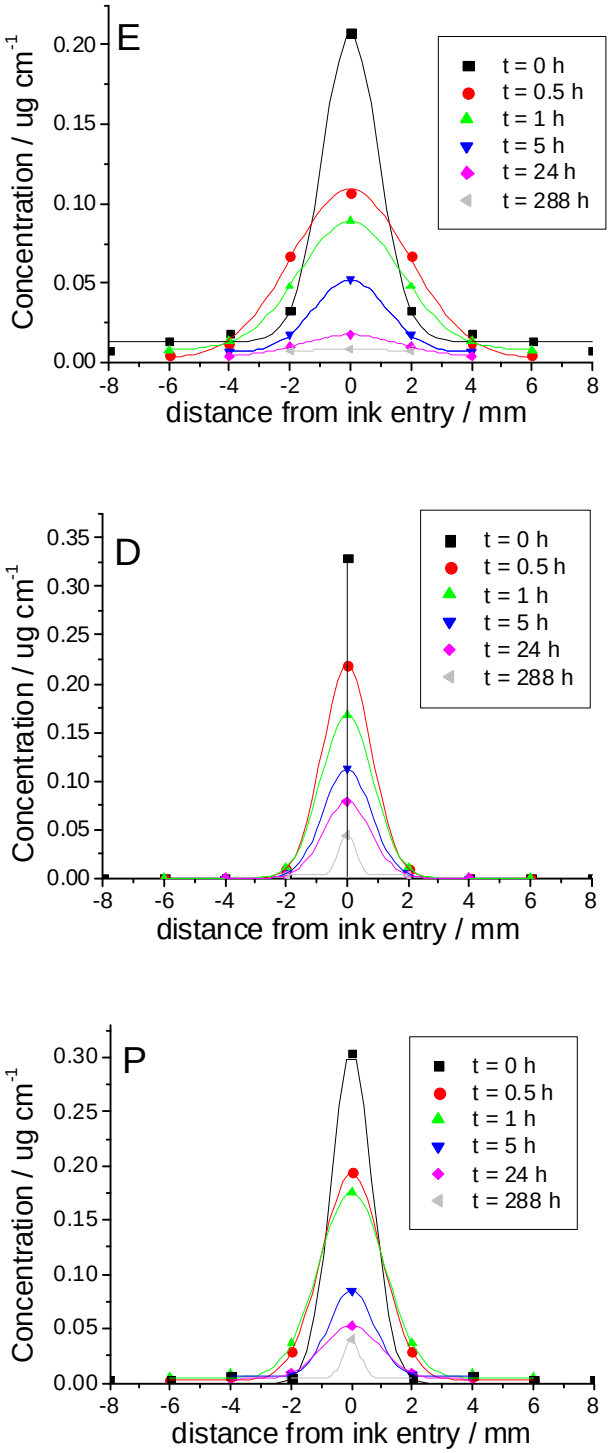


Figure 4.51 Diffusion [ug/cm] of the solvents ethoxyethoxyethanol (E), dipropylene glycol (D), phenoxyethanol (P) away from the ink stroke (0 mm) at different times t after applying the stroke to the paper. The curves represent Gaussian diffusion fits. The error ranges (not shown in the plots) are between 20 and 50% of the small quantities involved.

In figure 4.52, the concentrations of E, D and P as measured at a distance of 2 mm from the stroke are plotted as functions of time. These curves reflect the complementary processes of evaporation and diffusion; the solvents migrate into the paper and evaporate at the same time.

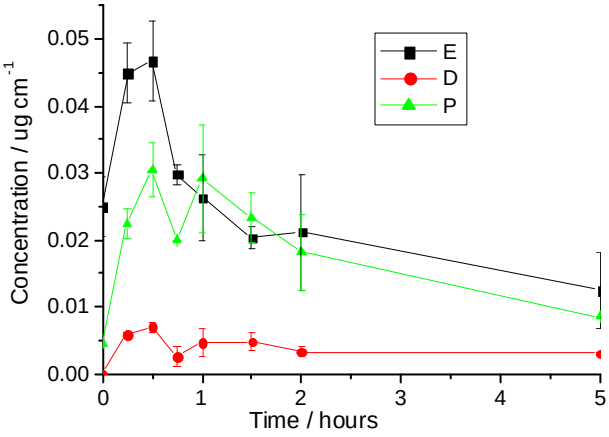


Figure 4.52 Solvent loss by evaporation and diffusion [µg] determined for ethoxyethoxyethanol (E), dipropylene glycol (D), phenoxyethanol (P) from GC/MS measurements made at a distance of 2mm next to the ink entry, as functions of the time elapsed since apposition of the ink to the paper.

The fact that diffusion plays such an important role in the disappearance of some solvents from the stroke invites some consideration about the sampling of the stroke. Other researchers in their analyses [Lociciro et al., 2004] have tried to cut the ink stroke with as little paper as possible, so as to avoid interference of paper with the analysis. However, our results about diffusion indicate that as much paper as possible should be cut out with the stroke so that a meaningful amount of solvents can be collected by extraction. There is an upper limit of 10 x 2 mm, simply because larger pieces cannot adequately be immersed for extraction into 10 µl solvent.

A small extraction experiment was performed to demonstrate the effect of paper size. A stroke of 1 cm was cut out with as little paper as possible, and then extracted. Compared with the mean values obtained when extracting the stroke on 10 x 2 mm of paper, these results indicate that only 29 % of E, 74 %

of D and 66 % of P were recovered from the minimum size piece. This fact alone would account for a significant loss in quantification sensitivity.

Contamination

Possible contamination of old strokes through solvent migration from fresh strokes on adjacent sheets of paper, was also tested (Fig. 4.53). It was observed that solvents from a fresh stroke (t = 0) can very efficiently migrate to adjacent sheets of paper in a pile.

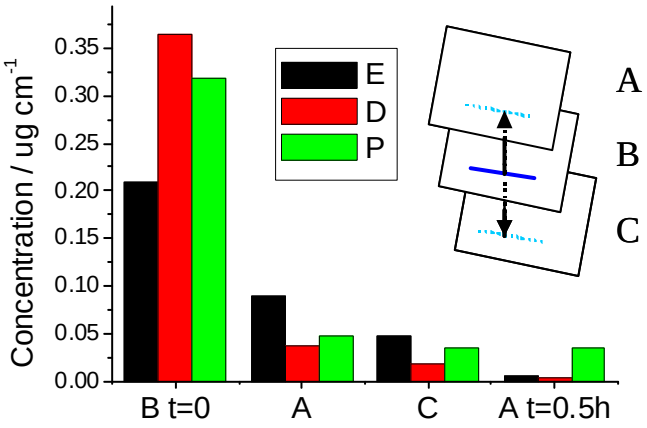


Figure 4.53 Migration of the solvents ethoxyethoxyethanol (E), dipropylene glycol (D), phenoxyethanol (P) from an ink entry to an adjacent sheet of paper during a 15 min contact (values correspond to single measurements): quantities in the stroke at t=0 (B), quantities in a sheet of paper placed over B (A), quantities in a sheet of paper placed under B (C), and quantities in a sheet placed over B (A) 0.5 hours after apposition of the ink entry on B.

Our measurements show that about 0.025 to 0.075 µg of the solvents E, D and P did migrate into the adjacent sheet of paper during the 15 min contact. Even when the contact was made as late as half an hour after applying the stroke, more than 0.025 µg of P still migrated into the adjacent paper. The quantities of solvent involved in this migration exceeded those found in a stroke after two weeks, so that conversely, contamination of a stroke by migration must be taken into account for the dating of ink entries by solvents quantification.

In absolute values, the quantities diffusing and migrating into the paper are very low. Since the paper structure is uneven, diffusion patterns are not very reproducible, and measuring errors was rather high (up to 50% relative mean standard deviation). These fluctuations in the diffusion and migration patterns act so as to increase the error in measurements of the amount of solvents in ink entries.

Testing of the method on entries of known age and known composition

Ink entries of known age that had been held in a file folder were provided by the BKA (Wiesbaden), and analysed by GC/MS. Pieces of paper with 5 cm entries were cut, introduced into separated glass vials, and send to our laboratory in January 2004. The entries originated from five ballpoint pens, and were created on the 27th of November 1990, the 16th of August 1999 and the 26th of November 2003, always on a different type of paper. A fresh entry was drawn for comparison on the 10th of August 2004. The five ballpoint pens contained the solvent P (b4, b5, b6, b7, b8), and four of them contained PE and BA (b5, b6, b7, b8). Two of them additionally contained the solvent HG (b7) or BE (b8). In the chromatogram of the entries drawn in November 2004, only P and PE were still found in small quantities (1.9 to 7.5 % of the initial values). After 4 years, only the entries from ballpoint pen b8 entries still contained a little P, but after 14 years, no solvent was recorded in any of the ink entries. The quantitative results are shown in Table 4.9. The ballpoint pen entries were stored in a file folder before they were sent for analysis in small glass vials. Analysis was performed seven months after the ink entries were sent. Entries from ballpoint pens b7 and b8 were drawn on 4th of May 2004, held in a file folder, and analysed 3 months later. Quantitative analysis gave values of 0.25% (b7) and 0.32 % (b8) of the initial values, i.e. less than the values found for the 9 months old entries of November 2003. This can be explained by the influence of two variables in the storage conditions: the BKA entries were held in a file folder with many other sheets of paper containing ink entries, and they were kept in a tightly sealed container for a period of about two months after their application to paper. Contamination through solvent migration in the file folder is likely, and

if the atmosphere in the glass vial was saturated with evaporated solvent, then drying could have been much slower. The 3 months old entries were kept in a file folder with two blank sheets of paper between any sheets with entries, so that drying was possible through diffusion into other sheets of paper. In addition, these entries were made on different type of white print paper.

Quantity of phenoxyethanol [µg]				
Pen	**2004 (t=0)**	**2003**	**1999**	**1990**
B4	0.032 (100 %)	0.002 (7.5 %)	0	0
B5	0.383 (100 %)	0.010 (2.7 %)	0	0
B6	0.573 (100 %)	0.017 (3.0 %)	0	0
B7	0.412 (100 %)	0.031 (7.4 %)	0	0
B8	0.663 (100 %)	0.013 (1.9 %)	0.004 (0.6 %)	0

Figure 4.9 Results of GC/MS quantitative analysis for the solvent P contained in ink entries of five ballpoint pens provided by the BKA; entries one year old still contained 1.9 to 7.5 % of the initial quantities of P. Only the entries from ballpoint pen b8 still contained P after 4 years (0.6 %), and none of the entries contained any solvent after 14 years.

These results demonstrate the importance of the storage conditions for the quantitative analysis of solvents. They also show that the percentage of P still found in the entries of November 2003 varied considerably between pens, revealing a differential drying behaviour that cannot be explained, merely in term of different initial amounts of solvent in the entries.

Il est bon de savoir que l’utopie n’est rien d’autre que la réalité de demain et que la réalité d’aujourd’hui était l’utopie d’hier

It’s good to know that the utopy is nothing but the reality of tomorrow and that the reality from today was the utopy from yesterday

Le Corbusier

5 INTERPRETATION

5.1 Summary and forensic interpretation

Chemical analysis of ink is performed with two major aims. On the one hand, it is a matter of classifying the different inks available on the market, and of possibly identifying the ballpoint pen which gave rise to the transfer (*inference of source*). On the other hand, it is an important task for dating purposes to determine the age of the ballpoint entry (*time of contact*). The information gathered is supposed to settle the question whether a fraud is present or not.

Static dating is based on industrial changes in ink composition that occur over time. Two conditions have to be met to allow the identification of a particular type of ink introduced timely on the market. (i) One must have access to a comprehensive database of the ballpoint pen inks existing or having existed on the market. This should cover a broad geographic region (ideally worldwide) and a broad time span (ideally all the way back to market introduction). (ii) Identification of a given type of ink according to a given analytical method must be reliable over time. Solvent drying and dye fading may sufficiently alter an ink so that the characteristics of an ink entry made at a given time will not match the characteristics of the same ink at time t=0 (false negative), or rather match those of another ink at time t=0 (false positive). Thus, the aging behaviour of the inks contained in the database should be studied with great care in order to more particularly avoid the possibility of false positives.

Dynamic dating is based on the determination of aging parameters and kinetics. If these parameters are reproducible under given circumstances, it is possible to determine the age of an entry and, thus, the time when the entry was apposed on the document. Similar issues are encountered when determining the time of death or the age of a person in forensic medicine. This is a rather hard challenge in most cases, because aging is influenced by many other factors apart from time that may accelerate or quench the given

processes. It is essential that the dating methods be validated and their reliability established before practicing them and presenting their results in court.

The factors influencing the aging of an ink can be classified in two main groups: the storage conditions and the initial ink composition (Figure 5.1). Time causes aging, which leads to quantitative and qualitative changes in the composition of ink. These changes can be followed by analytical means, and may give indications as to the time elapsed between application of the ink to paper and the measurements. Since external factors (storage conditions) and internal factors (composition of ink) decisively influence the aging processes of ink, they have to be known and taken into account when interpreting the analytical results.

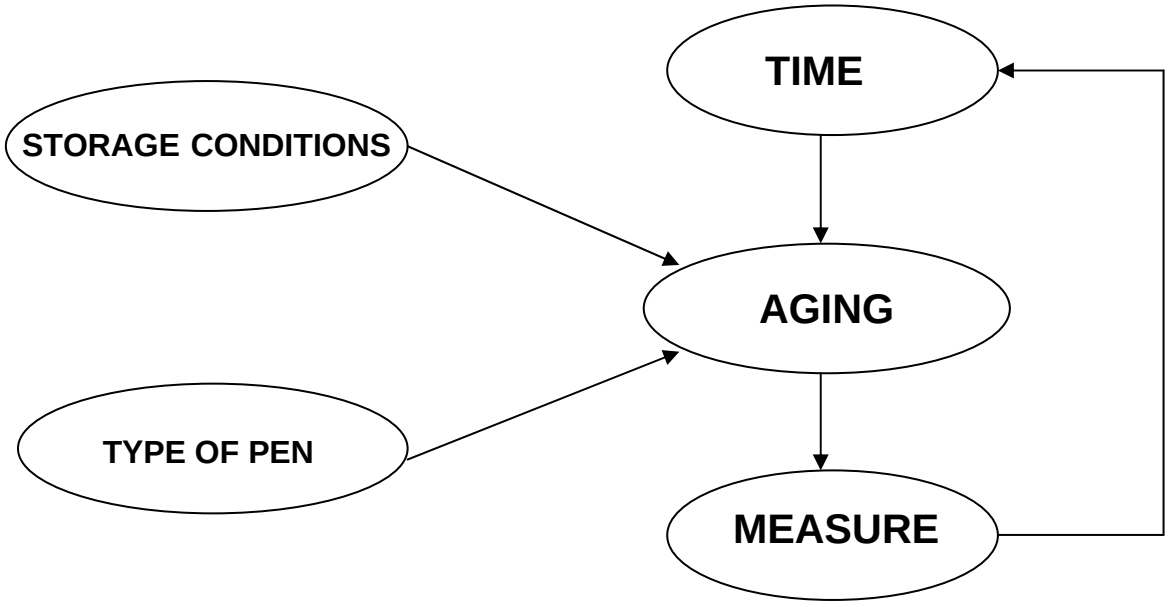


Figure 5.1 This diagram represents the effect of time on aging, an effect that is governed by two main groups of variables: the storage conditions and the composition of an ink entry. Aging leads to changes in ink composition that can be analytically determined. The outcome of such measurements gives an indication as to the age of an ink entry.

The fading of dyes in the ink and the loss of solvents from the ink are major aging mechanisms. Dye fading (Fig. 5.2) strongly depends on the storage conditions, and more particularly on the intensity and wavelength of the incident light. Therefore, any exposure to light, even only to examine the document (e.g. optical comparison using a set of light sources with different wavelengths ranges) should be avoided or controlled. Temperature (which will rise at the paper surface under incident light), humidity and substrate chemical composition also have measurable effects on aging by dye fading. The type of pen that was used is important, since details of ink composition (dye structure, dye mixtures, additives) may cause acceleration or quenching of the aging processes and since the size of the ballpoint and the physical properties of the ink will affect the thickness (concentration/surface) of the ink layer produced.

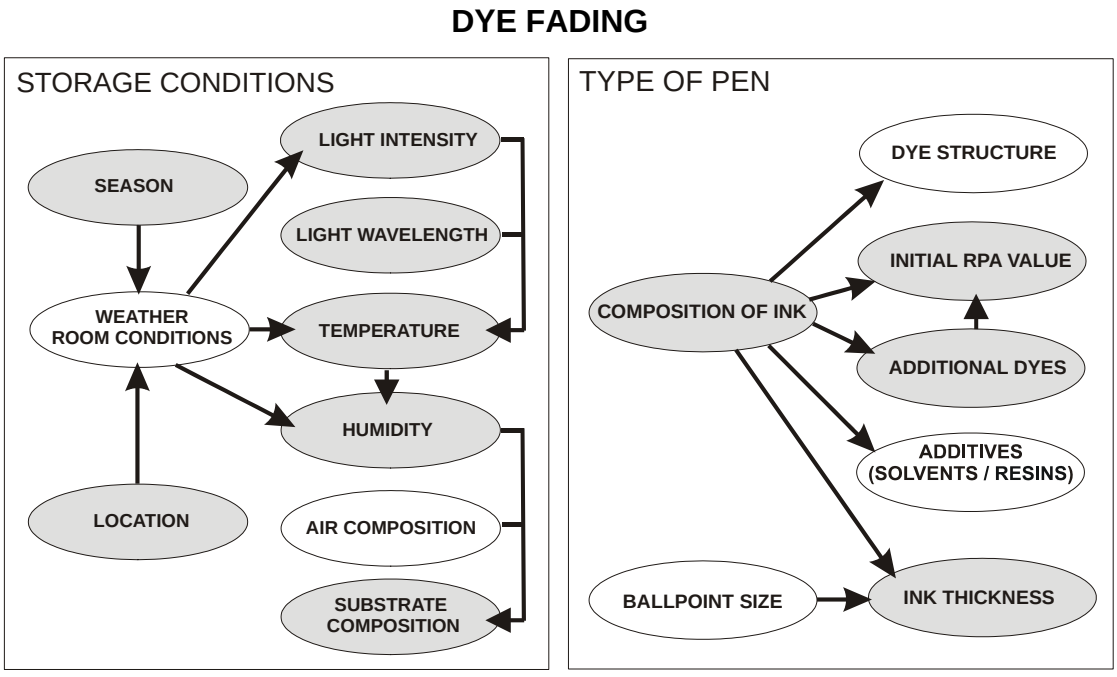


Figure 5.2 The factors influencing the fading of dyes in ink on paper are classified in two main groups: the storage conditions constituting indirect factors (to the left) and the type of ballpoint pen and ink constituting direct factors (to the right). Arrows and their direction identify the factors of influence. Grey shading identifies factors evaluated in the present work.

Solvent drying (Fig. 5.3) strongly depends on temperature, humidity, air flow, and substrate physical properties so that the storage conditions are an essential factor in the kinetics of the process. The presence of porous or non-porous adjacent materials is important too. Ink composition will affect drying, since the evaporation and diffusion processes depend on the type and surface area (concentrations) of solvents and may also significantly change with the presence of other solvents, additives or traces such as fingerprints and greasy stains.

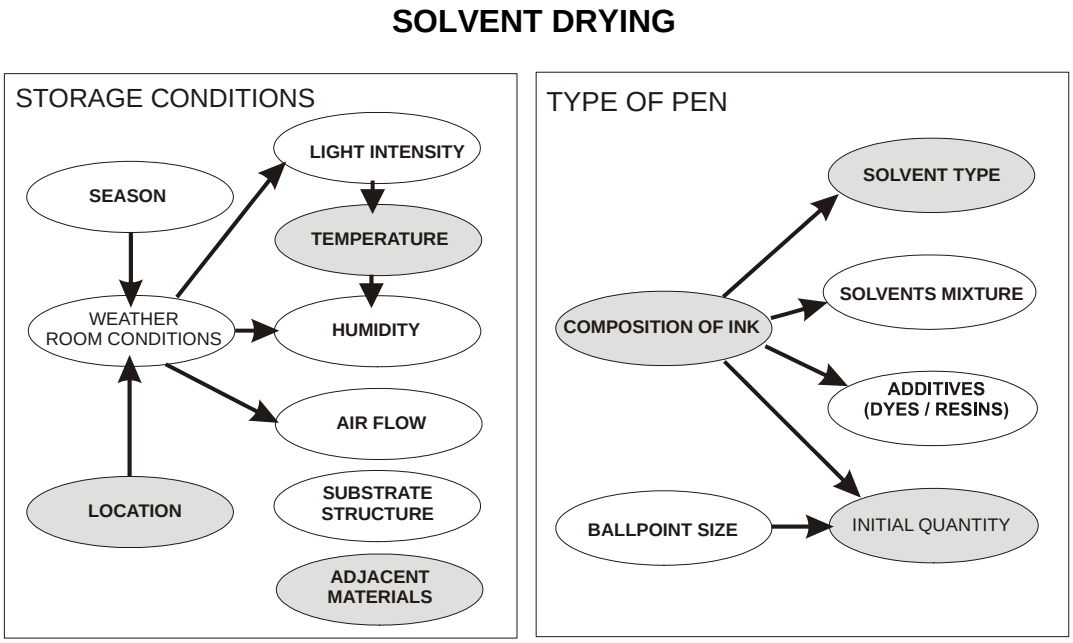


Figure 5.3 The factors influencing the drying of solvents in ink on paper are classified in two main groups: the storage conditions constituting indirect factors (to the left) and the type of ballpoint pen and ink constituting direct factors (to the right). Arrows and their direction identify the factors of influence. Grey shading identifies factors evaluated in the present work.

These factors are known and their influence has been studied in other domain. The results of the present work yield improved knowledge of the extent of effects of these factors on the aging processes. It represents a fundamental study and quantitative evaluation of the aging processes of ballpoint ink generally missing from other work done on this subject so far. It also

emphasises such phenomena as a possible contamination of evidence by solvent migration from one sheet of paper to another.

Ink aging is a very complex process, and interpreting this type of analytical evidence is not a matter of casual trials but, to the contrary, should be a careful undertaking including a serious evaluation of the method used [Starrs, 1994]. The first, and most important, step in the interpretation of evidence is that of *asking the right question* before trying to answer it [Inman and Rudin, 2001]. According to the theory of bounded rationality introduced in 1975 by H. A. Simon [Gigerenzer and Selten, 1999], the decision-making process is described as a search process guided by aspiration levels. Decision alternatives must be found and dynamically adjusted to the situation. Limitations mainly arise because unknown variables and wrongful aspirations may lead to erroneous conclusions [Risinger et al., 2002]. One must imperatively answer this question: can a decision reasonably be taken under the conditions on hand?

5.2 Applications and limitations in forensic cases

Robertson and Vignaux wrote [1995] that *an ideal piece of evidence would be something that always occurs when what we are trying to prove is true and never occurs otherwise*. In a Bayesian approach, this would mean that the evidence is always observed when the hypothesis is true, and never when the hypothesis is false. In reality, practically no evidence is ever as easy to interpret, and the following probabilities have to be determined in order to evaluate the strength of a piece of evidence:

- the probability to observe the evidence E (e.g. RPA values) provided the ink entry has been made at a time (t_2)
- the probability of this same evidence provided the ink entry has been written at a later (posterior) time (t_1).

These probabilities depend on the groups of factors listed above. Under controlled storage conditions and with known initial composition, it would be possible to determine an aging curve for the given set of conditions (e.g. the laboratory conditions) and determine the probabilities.

At first, we will examine the difficulties of interpreting the fading of dye over time. The RPA values are used as an indication of the extent of the fading of a dye. At time t = 0 when the ink is applied on paper, the RPA_{372} values for 31 ballpoint pens purchased in Germany were obtained by LDI-MS, and a probability histogram was generated involving steps of 5% (Figure 5.4).

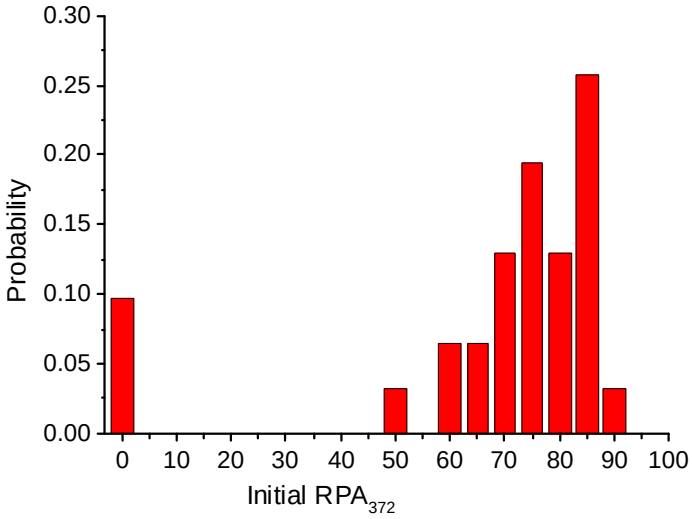


Figure 5.4 Probability histogram (on a scale of 5% steps) for the initial RPA_{372} values obtained by LDI-MS for the dye MV in 31 blue ballpoint pens inks. There is 26 % probability that a pen has an initial value of 85%, a 10% probability that a pen does not contain MV and a 0% probability that a pen has initial RPA_{372} values as low as 55% or as high as 95%.

The initial composition of an ink and, more particularly, the initial RPA value of the dye MV (RPA_{372}) vary greatly between ballpoint pens. When MV was present (probability of 0.9), then the RPA_{372} values were found between 50 and 90%. The highest probabilities lay at 0.26 for RPA_{372} = 85% and 0.20 for RPA_{372} = 75%. Two pens gave extreme values of 50% and 90%, respectively (with probabilities of 0.03 among the 31 values). Aged RPA_{372} values were obtained for only a few pens (i.e. Bic1a, Bic1b, Herlitz and Parker).

If the forensic scientist was confronted during interpretation with RPA_{372} values of 0%, 60% or 90%, what could he possibly conclude about the age of the entry (see Table 5.1)?

RPA_{372}	Possible sources	Probability
0%	- entry at t = 0 - entry at t = 148 days (daylight, e.g. Herlitz) - entry at t = 320 days (daylight, e.g. Bic1b) - entry at t = 1032 days (daylight, e.g. Bic1a) - other	- 0.1 - unknown - unknown - unknown - unknown
60%	- entry t = 0 (e.g. Bic1b) - entry at t = 11 days (daylight, e.g. Parker) - entry at t = 2years (dark, e.g. Bic1b) - entry at t = 2 years (dark, e.g. Herlitz) - other	- 0.06 - unknown - unknown - unknown - unknown
90%	- entry at t = 0 (e.g. Bic1a) - entry at t =1000 days (dark, e.g. Bic 1a) - other	- 0.03 - unknown - unknown

Table 5.1 Summary of possible sources for obtaining RPA_{372} values of 0%, 60% or 90% by LDI-MS analysis of ballpoint ink entries. A probability of observing the evidence given the ink entry has been made at a time t is available only for t = 0.

- RPA_{372} values of 90% were measured only for the BIC1a ballpoint pen at time t = 0 and at time t = 3 years of storage in a dark cabinet.
- RPA_{372} values of 60% were obtained for two ballpoint pens at time t = 0, for the Parker entries at t = 11 days exposure to daylight, and for the Herlitz entries at t = 2 years storage in the dark.
- A RPA_{372} value of 0% was attributed if no signal corresponding to MV was present in the spectra. Three pens had no MV in their initial composition. Also, MV was no longer found in the BIC1a entries after 1032 days, in the BIC1b entries after 320 days, and in the Herlitz entries after 148 days natural aging in daylight.

The thickness of the ink layer on paper also plays a role in the kinetics of the aging processes. It is essential, therefore, that more studies be carried out to evaluate the possible hypotheses and their associated probabilities. This is a highly time and resource consuming task. In practical cases, any information that can be gathered as to the type of pen that was used, and the storage conditions, may be helpful in excluding some possibilities and change prior probabilities. The amount of light to which an ink entry is exposed is an essential parameter. For example, when a RPA_{456} value of 50% is obtained for the dye EV of a BIC1a ballpoint entry, four possible sources have been identified for this particular pen in terms of exposure to light:

- naturally aged in winter 2001/02 during 168 days
- naturally aged in summer 2002 during 72 days
- naturally aged in summer 2004 during 48 days
- artificially aged during 15 days

When the type of pen is known, but no information is available as to the storage conditions, then all these hypotheses about possible sources have the same probability, and no conclusion can be advanced. Depending on ink composition, dyes on paper stored in the dark aged very slowly, and their RPA_{372} values may well lie within the range of initial values at time t=0. Therefore, no conclusion would be possible if the ink composition at time t=0 was unknown.

The same kinds of problems arise in the concentration measurements of solvents in ballpoint pen entries. At time t = 0, the initial concentrations of solvent P in ink entries from 31 blue ballpoint pens have been determined (Fig. 5.5). There was a wide variation in the values obtained: they ranged from 0 to 0.65 µg/cm. The distribution probabilities were found from 0 and 0.16. Therefore, knowledge of initial ink composition is imperative for any attempt at dating an entry on the basis of the solvent concentration.

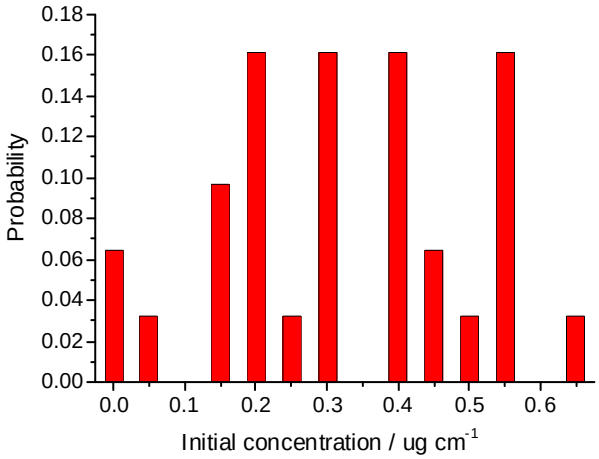


Figure 5.5 Probability histogram (scale with 0.05 steps) for the initial concentration [µg/cm] of solvent P determined by GC/MS in the inks from 31 blue ballpoint pens. There is a 16 % probability that a pen stroke contains an initial solvent quantity of 0.2, 0.3, 0.4 or 0.5 µg. Many of the values have been extrapolated from the SCAN chromatogram, and constitute single values (the error for single values may be rather large because of the fibrous character of the paper).

Considering the laboratory conditions prevailing in this work, the probability of observing a concentration above 0.1 µg/cm in entries made at a time of t = 24 hours is definitely higher than that of observing it in entries made at a time of t = 2 years. One has to be careful as to storage conditions, for instance because of the possibility of contamination (in a notebook or file folder) or the suppression or reduction of drying processes in containers tightly sealed (glass vial) or semi hermetic (plastic cover). Ink entries in this work were drawn as straight lines, allowing solvents to diffuse away from the stroke. A questioned document will most probably carry a text with curved line from an alphabet. For example in the letter “o”, the solvents will diffuse to some extent away from the letter and partly inside the ring. This might increase the quantities of solvents found in such a letter compared to a straight line of the same length (Figure 5.6).

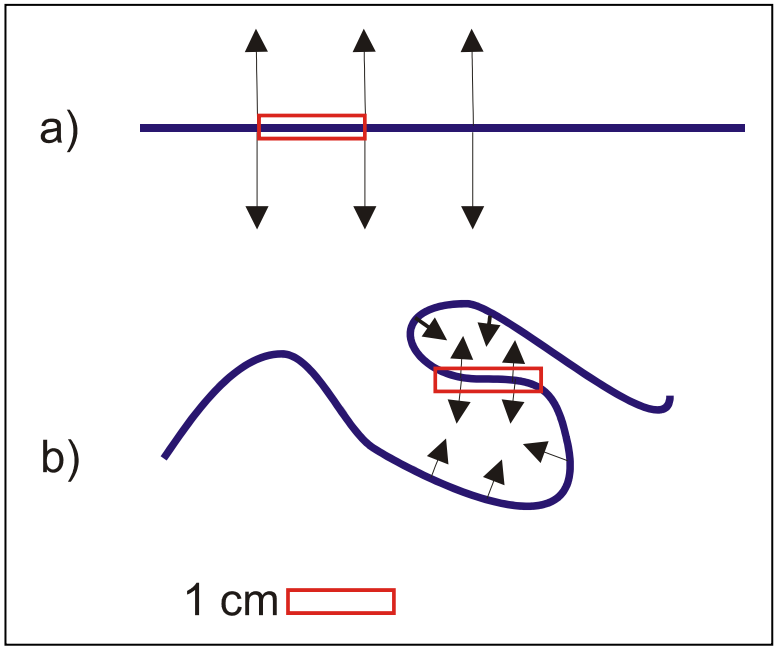


Figure 5.6 Solvents diffusion from two ink entries: (a) diffusion away from a straight line, (b) diffusion inside the loops of a curved line. The solvent concentration might significantly be larger in 1 cm of (b) compared to 1 cm of (a).

Temperature is also important; thus, with a Parker ballpoint pen stroke on the Igepa printer paper, it was equally probable to find a concentration of 0.1 µg/cm for an entry made at a time of t = 24 hours and an ambient temperature of 20℃, as for an entry made at a time of t = 2 hours and a temperature of 60℃.

A particularly important, but problematic variable is the quantity of ink extracted. A signature often is very small, not straight, and of non uniform line quality and thickness. Hence, it is not possible to repeat the analysis a number of times, and it is difficult, too, to cut a number of exactly 1 cm entries of identical thickness. One way to resolve this reproducibility problem is the use of a mass independent value such as the concentration of drying solvent, divided by the concentration of a persistent compound inherent to the ink. Aginky [1996] proposed a sequential extraction ratio value. Such ratios have to be constant for any given pen, or defined for each pen in a database. The influence of substrate structure (paper type) on the drying process should not be underestimated, as their porosity can differ quite widely within a same sheet of paper (pores diameter between 0.05 – 10 µm). Molecular (Fickican) diffusion, Knudsen

diffusion, surface (pore) diffusion, capillary condensation, physisorption (absorption and adsorption), chemisorption, migration and evaporation will all be influenced by the porous structure of the paper, the fibers and eventual additives.

The definition of threshold values has been proposes as a possibility to solve the problem of unknown variables. For example, Aginsky [1996] first proposed boundaries to be used when determining a particular aging parameter (e.g. decrease of drying rate):

- fresh inks (less than five, eight or twelve month old)
- old inks (older than six months for entries stored at room temperature)

This approach has been used by scientists for expertise in Austria and Southern Germany, but no information has been officially published about the development of new methods. If such interval values where valid for given storage conditions and ballpoint pen that could have been used, it would then be possible to claim, for example, that a value 0.1 µg/cm can only be observed when the entry was made within the two prior weeks, but never when an entry is older than two years. Considering the great variability in the drying of solvents caused by identified aging parameters, such threshold values will never be applicable to every possible case. Some additional information about the pen and the storage conditions will always be necessary, and a significant uncertainty will persist.

Dating methods involving fading of dyes or drying of solvents have to be validated and their reliability established while taking into accounts all of the factors of influence, identifying all possible sources, and evaluating the probabilities of all hypotheses. Strictly, these methods should only be applied when comparing two ink entries made with the same pen and in the same thickness, on the same sheet of paper (of uniform quality), and stored under the same conditions, without sources of contamination (all factors must be controlled). Then, a difference in RPA values of dyes or in concentrations of solvents would suggest a difference in age.

5.3 Simulation of absolute dynamic dating

The statistical data required to evaluate probative values of dating evidence were not available, however, subjective estimates of these probabilities were attempted while using results from the present work. It was the aim of this exercise to give an example of how such values might be used in a forensic case if the objective data were available, and what information they could provide. It was possible in this way to perform a simulation of the absolute dynamic dating procedure. The drying curves (Fig. 4.46) and the data (Table 4.9; Fig. 5.5) presented earlier in Chapter 4 for solvent drying were used to extrapolate a plausible approximation of the probability values for solvent concentrations in ballpoint ink entries. In the solvent drying curves (fig. 5.7) measured by GC/MS, three visible aging phases could be distinguished covering the first few hours (0 to 3 hours), the first few days (3 to 48 hours), and the time beyond two weeks (> 336 hours).

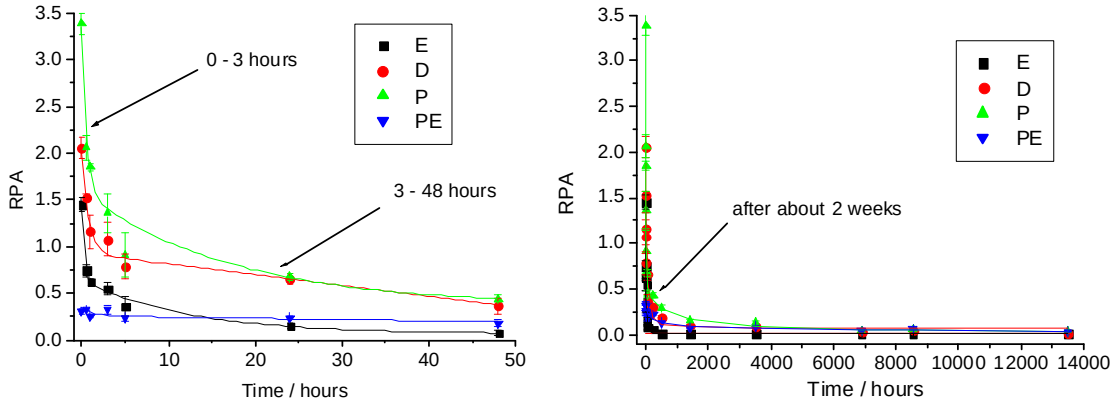


Figure 5.7 Drying curves obtained by GC/MS for four ballpoint solvents (E: ethoxyethoxyethanol, D: dipropylene glycol, P: phenoxyethanol, PE: phenoxyethoxyethanol) from Parker ballpoint pen entries. The Relative Peak Areas (RPA) plotted as functions of time are proportional to the solvent concentration. Drying slowed down considerably with time, and three stages can be distinguished: 0 to 3 hours, 3 to 48 hours and beyond two weeks.

These limits are not relevant for the dating of ink in real cases, unfortunately, because the time needed for a judge to order an expertise usually is more than one month. In the data covering the time beyond a few weeks, measuring errors

increased when analyzing the low solvent quantities found in the corresponding ink entries, but it is precisely these results that are pertinent to the dating of ink.

Objective statistical data about the possible age of an ink entry could be of the form of, or close to, the data proposed in Table 5.2 (for any type of pen and for times beyond ten years). It was assumed that the ink entries had not been kept in tightly sealed containers (drying actually occurred) and that temperature did not exceed a certain value (no accelerated aging). Paper type and exact storage conditions were disregarded when choosing these subjective values. The concentrations were divided into four ranges assumed to cover all possible cases S: c_1 = 0.1 to 1 mg/cm; c_2 = 0.01 to 0.09 mg/cm; c_3 = 0.001 to 0.009 mg to cm and c_4 = 0 mg/cm, the set of all possibilities being S= {c_1, c_2, c_3, c_4}. The limit of detection was set at 0.001 mg/cm. Logically, if an entry contained high concentrations of solvent, it was more probably fresh than old, while low concentrations more likely indicated an older entry (see also Table 4.9).

A probability of 0 means that under the given conditions, the case c_i is never encountered. Thus, if c_1(≥ 4 months) = Φ_1 and p(Φ_1) = 0, then c_1(≥ 4 months) is never encountered:

$$p(c_1 \mid t \geq 4 \text{ months}) = 0 \qquad \text{Eq. (5.1)}$$

In other words, the concentration range c_1 was never found in an ink older than four months. On the other hand, if c_2(4 months) = Φ_2 and p(Φ_2) = 0.8, then c_2(4 months) is encountered 8 of 10 times:

$$p(c_2 \mid t = 4 \text{ months}) = 0.8 \qquad \text{Eq. (5.2)}$$

This means that the concentration range c_2 was found in 80% of the 4 months old ink entries. The added probability of S covers all four sets of concentrations, and for a particular time/age condition is equal to 1:

$$p(c_1 \cap c_2 \cap c_3 \cap c_4 \mid t=0) = 1 \qquad \text{Eq. (5.3)}$$

SUBJECTIVES CONDITIONAL PROBABILTITIES FOR SOLVENT DRYING DATA					
Age of entry	**c_1**	**c_2**	**c_3**	**c_4**	**S**
(condition)	**0.1 - 1 mg/cm**	**0.01 - 0.09 mg/cm**	**0.001- 0.009 mg/cm**	**0 mg/cm**	**all possibilities**
t = 0	0.91	0.03	0	0.06	1
1 day	0.8	0.14	0	0.06	1
1 week	0.47	0.47	0	0.06	1
1 month	0.05	0.85	0.04	0.06	1
2 months	0.03	0.8	0.11	0.06	1
4 months	0	0.8	0.12	0.08	1
6 months	0	0.45	0.45	0.1	1
1 year	0	0.2	0.5	0.3	1
2 years	0	0.05	0.45	0.5	1
3 years	0	0	0.2	0.8	1
4 years	0	0	0.1	0.9	1
5 years	0	0	0.05	0.95	1
10 years	0	0	0.01	0.99	1

Table 5.2 Subjective conditional probabilities proposed for the drying of a solvent from ballpoint entries. It was assumed that the ink entries were not kept in tightly sealed containers (drying actually occurred) and that temperature did not exceed a certain value (no accelerated aging). Paper type and exact storage conditions were disregarded when choosing these values. They were divided in four concentration ranges assumed to cover all possible cases: 0.1 to1 mg/cm; 0.01 to 0.09 mg/cm; 0.001 to 0.009 mg/cm and 0 mg/cm. The limit of detection was set at 0.001 mg/cm.

Having subjective conditional probability values at disposition, it was then possible to check the probability of observing the evidence c_i (concentration in $mg \cdot cm^{-1}$) given the ink entry considered had been made at time t_1 (hypothesis of the prosecution (H_p): suspected age of entry, generally younger than age written on document), compared to the probability of this same evidence given the ink entry had been written at a prior time t_2 (hypothesis of the defense (H_d): claimed age of entry or age written on document), by using the likelihood ratio definition:

$$LR = P(c_i \mid H_p) / P(c_i \mid H_d) \qquad \text{Eq. (5.4)}$$

The results in Table 5.3 are more probable to be encountered in real forensic cases, because it is more relevant to compare values older than one month. The likelihood ratio is an indication of the strength of the evidence in supporting our two hypotheses: hypothesis H_p (entry made at time t_1) as compared to hypothesis H_d (entry made prior to that time). Values below 1 support the defense hypothesis H_d, while values above 1 support the prosecution hypothesis H_p.

The larger the value of LR, the more probable is the fact to observe the analytical results (c_i) given H_p is true. To the contrary, the smaller the value of LR, the more probable is an observation of the analytical results (c_i) if H_d is true. An LR value of 1 means that the two hypotheses have identical probabilities. The LR values became larger when the time differences compared by the hypotheses were larger (e.g. up to 50 for Δ = 9 years, and down to 1.11 for Δ = 1 year). In cases where one of the hypotheses H_i is demonstrated to be impossible given the evidence c_i ($p(c_i/H_i)=0$), the analytical scientist is able to reach a clear conclusion as to the age of an ink entry without any statistical treatment (ideal case). In all other cases ($0< LR <\infty$), only indications as to the probability of evidence for a given hypothesis to be true can be obtained. This may be helpful in rendering a judgment, but in no way constitute proof for a fraud in itself. The probabilities and LR values presented above are subjective estimates, and could substantially diverge from calculated statistical values. They were only used as an example illustrating which data is necessary to date a document, and how to use them for this purpose. Depending on the objective statistical data available for an aging parameter (e.g. RPA or concentrations), the dating of document will be efficient (i.e. the LR values are very large or very low) or inefficient (i.e. the LR values are close to 1). For this reason, the adequacy of the aging parameters for dating purposes has to be checked quite carefully and early in the process of setting up a dating procedure.

$$LR = P(c_i|H_p)/P(c_i|H_d)$$

Age of entry	**Evidence E**			
H_p= 2 months	**c_1**	**c_2**	**c_3**	**c_4**
H_d:	*0.1 - 1 mg/cm*	*0.01 - 0.09 mg/cm*	*0.001- 0.009 mg/cm*	*0 mg/cm*
4 months	H_d not possible	1	0.92	0.75
6 months	H_d not possible	1.78	0.24	0.60
1 year	H_d not possible	4.00	0.22	0.20
2 years	H_d not possible	16.00	0.24	0.12
5 years	H_d not possible	H_d not possible	2.2	0.06
10 years	H_d not possible	H_d not possible	11	0.06

Age of entry	**Evidence E**			
H_p= 1 year	**c_1**	**c_2**	**c_3**	**c_4**
H_d:	*0.1 - 1 mg/cm*	*0.01 - 0.09 mg/cm*	*0.001- 0.009 mg/cm*	*0 mg/cm*
2 years	H_p+ H_d not possible	4.00	1.11	0.60
3 years	H_p+ H_d not possible	H_d not possible	2.50	0.38
4 years	H_p+ H_d not possible	H_d not possible	5.00	0.33
5 years	H_p+ H_d not possible	H_d not possible	10.00	0.32
10 years	H_p+ H_d not possible	H_d not possible	50.00	0.30

Age of entry	**Evidence E**			
H_p= 2 years	**c_1**	**c_2**	**c_3**	**c_4**
H_d	*0.1 - 1 mg/cm*	*0.01 - 0.09 mg/cm*	*0.001- 0.009 mg/cm*	*0 mg/cm*
3 years	H_p+ H_d not possible	H_d not possible	2.25	0.63
4 years	H_p+ H_d not possible	H_d not possible	4.50	0.56
5 years	H_p+ H_d not possible	H_d not possible	9.00	0.53
10 years	H_p+ H_d not possible	H_d not possible	45.00	0.51

Age of entry	**Evidence E**			
H_p= 3 years	**c_1**	**c_2**	**c_3**	**c_4**
H_d:	*0.1 - 1 mg/cm*	*0.01 - 0.09 mg/cm*	*0.001- 0.009 mg/cm*	*0 mg/cm*
4 years	H_p+ H_d not possible	H_p+ H_d not possible	2.00	0.89
5 years	H_p+ H_d not possible	H_p+ H_d not possible	4.00	0.84
10 years	H_p+ H_d not possible	H_p+ H_d not possible	20.00	0.81

Table 5.3 The likelihood ratio (LR) is an indication of the strength of the evidence in supporting our two hypotheses: hypothesis H_p (entry made 2 months, 1 year or 2 years ago) as compared to hypothesis H_d (entry made at a given posterior time). Values above 1 (red) support the prosecution hypothesis H_d, while values below 1 (blue) support the defense hypothesis H_p. A value of 1 means that the two hypotheses have identical probabilities. No values were calculated when one or both hypotheses are impossible (probability values of zero).

5.4 Proposed strategy to develop dating methods

As demonstrated above, the development of a dating method cannot be viewed as a simple laboratory task where a scientist would measure a few aging curves, and then apply them as an empirical rule to all ballpoint inks. Since the inference of sources and storage conditions clearly is an important issue, scientists that in the past have been confronted with this problem have suggested to develop relative aging methods in which entries from the same type of ink (identical source) stored under the same external conditions (identical storage conditions) are compared. Forensic cases meeting these requirements are very rare, and other methods had to be developed for absolute dating, independent of the type of pen and storage conditions.

Accelerated aging was first proposed by Cantu [Cantu, 1988] as a possibility to obtain an aging curve for an unknown ink entry. Document examiners still are engaged in a debate as to which kind of artificial aging factor to use (light, temperature), and whether artificial aging is really comparable with natural aging. A method developed by Aginsky and applied to forensic cases [Aginsky, 1998] made use of accelerate aging at elevated temperature (60℃) and sequential extraction. A segment of the aging curve was then calculated (from two ratio parameter values) and its slope provided indications as to the drying stage (i.e. the age). As explained above, higher temperature (like time) will lead to aging rates dependant on ink type, and it has never been formally demonstrated that threshold values fitting all ink entries actually exist.

Operational aging methods are based on solvent drying. Few details were supplied by the laboratories concerned, but it is relatively safe to allege that they are based on artificial aging and/or sequential extraction of solvent from a single ink entry [Aginsky, 1998; Andrasko, 2003; Bügler et al., 2005]. A percentage calculated from the results of two extractions (solvents, thermodesorption or SPME) diminishes with time. Threshold values have been fixed for all types of ballpoint inks.

Aginsky had cautioned that storage conditions should be known to be “normal” within an upper temperature limit (<25℃). The method does have the

advantage of being independent of the extracted mass (or entry thickness). However, no detailed studies about other factors such as type of paper, type of ink, adjacent materials, air flow or ink concentration all having a non-negligible influence on the aging process have been published. Up to now none of the ink dating laboratories has conducted blind testing for reasons open to conjecture. Refusal to publish details of the methods may be related to concerns about inter-laboratories validations that could reveal weaknesses in their methods, or to concerns to maintain exclusivity [Matley, 1994]. The latter concern is less probable, since sufficiently large number of forensic cases requiring dating arise every day around the world.

Since all document examiner dreams about having access to dating results, a strategy designed to establish dating methods will be proposed at the end of this Chapter. Many factors were taken into account, and the entire process will be divided into three compulsory phases and one optional final stage. One should never forget to ask the right questions and determine the right hypotheses before starting the process. This is a necessary condition for steady work in the right direction avoiding undesirable surprises.

Creation and maintenance of an ink database

As explained above, access to an extensive ink collection is decisive for both static and dynamic dating. Ideally, for every new ink composition on the market an ink sample and a pen should be obtained. To this end it will be necessary to develop and maintain very good contacts and arrangements with ink and pen producers in any given country or region; samples have to be obtained on regular basis. Only the US Secret Service (Washington, USA) and the LKA Bayern (Munich, Germany) are known to possess extensive sample and databases. The task of maintaining such a database is immense and delicate. At the end of last century, only five producers of ballpoint ink were counted in Europe [Andermann, 2001]. At that time, the ballpoint pen industries had only five distributors, which limited the number of different compositions.

Unfortunately, ballpoint pens now available in Europe are produced and originate from all around the world.

The laborious next step consists in analysing each new sample according to standardised methods. Several adequate mass spectrometric methods have been developed in the present work which will give direct information about the chemical composition of the ink (in contrast to comparative methods such as HPTLC or spectroscopy where the identification of substances is not always unequivocal). In few cases, ink formulations can be obtained directly from the producer. As dyes, solvents and resins age with time, i.e. their chemical compositions change to some extent, it might be useful to include data about components that do not age, such as inorganic trace elements or isotopic ratio. However, the techniques required for such analysis (ICP-MS, XRF, IRMS) are not always available for routine work, and their optimisation may take a considerable amount of time (reproducibility issues).

Date and location of market introduction are necessary criteria, and have to be included in the searchable library together with the data obtained by chemical analysis. Statistical tools can then be used to analyse the data stored and get an overview of market production (e.g. evolution in ink formulation with time or geography), and follow the tendencies (eventually identifying patterns).

Extensive study of the aging processes

The second task is not less laborious: the aging processes of selected substances have to be extensively studied under ordinary and unusual conditions. Some useful results were reported in the present work. Data for all types of inks of interest and for all possible storage conditions (taking into account temperature, air flow, paper movement, paper type, etc) should be obtained to build a database of aging curves. Time dependent (aging) factors (e.g. for solvents: artificial drying rate, sequential extraction ratios, or concentration) and the corresponding dating methods have to be selected and then tested.

A major problem apart from the amount of analytical work needed, probably is that of having the required storage room. Keeping ink entries from several types of pens on different kinds of paper in cabinets, pile of paper, file folders, plastic envelopes, or on a desk for many years is a logistic problem that could be a limiting factor in the selection of analytical techniques and factors to be studied.

The results presented above provide good indications as to the factors that are most decisive in forensic cases. Moreover, solvent drying occurs within smaller time spans than dye fading in the dark, and can for this reason be studied more extensively. It is certainly possible to divide the inks in groups having similar aging behaviour. Such considerations help to reduce the volume of experimental work. Sample size is a point that should be carefully decided before starting [Adock, 1997; Aitken, 1999]; the repeatability, reproducibility and measuring errors require early verification.

Once aging curves have been obtained, they can be compared. Thus, variability within curves for a single type of pen (under different storage conditions; Fig. 5.8); within curves for a single type of storage conditions (for different type of pen; Fig. 5.9); and within all curves (different storage conditions and ink composition; Fig. 5.10) may be determined through statistical means of comparison. This step is crucial for the development of aging methods, since it is at this stage that threshold values serving to generalize the dating process can be fixed. Extreme cases which do not fit within the limits have to be identified and defined as exceptions.

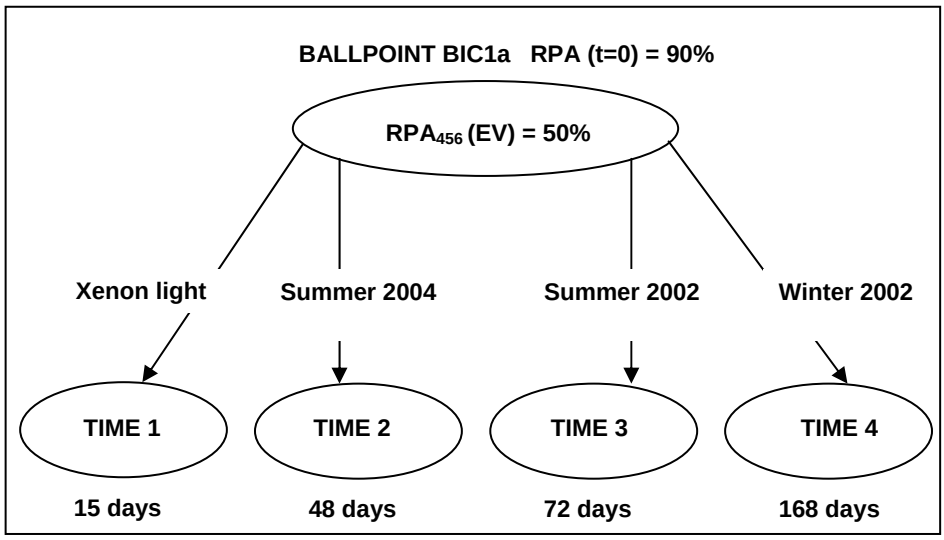


Figure 5.8 Inference of sources for a value of RPA_{456} = 50% in an ink entry produced by a given ballpoint ink. Four possible sources have been identified in this work for a BIC1a ballpoint pen: Xenon light, Summer 2004, Summer 2002 and Winter 2002.

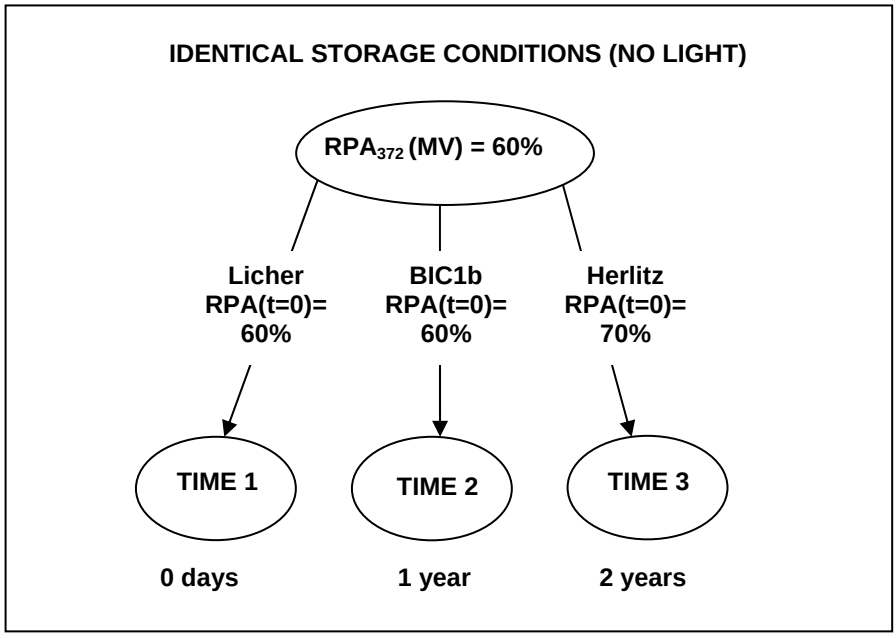


Figure 5.9 Inference of source for a value of RPA_{372} = 60% in an ink entry stored under given conditions. Three possible sources have been identified in this work: i.e. Licher, BIC1b, and Herlitz.

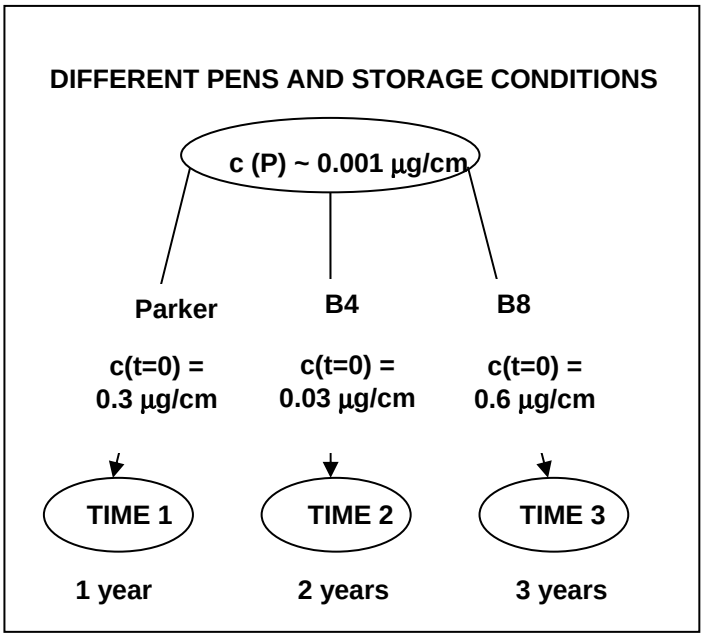


Figure 5.10 Inference of sources for a phenoxyethanol concentration of c (P) ~ 0.001 mg/cm in an ink entry with history of unknown storage conditions. Three possible sources with different initial concentrations of P have been identified in this work: i.e. Parker, pen B4 and pen B8.

After collection of sufficient amount of data covering a majority of cases, the probabilities will then be available of observing a given value, provided a given suggested hypothesis is true (e.g. the ink entry has been made at time t_1) or false (e.g. the ink entry has been made at another/posterior time), and the true strength of the dating evidence can be determined.

Intra-validation of the chosen methods

Once a method has been established within a given laboratory, it is imperative to perform blind testing and validation. This task should be performed with external, unknown samples, and an adequate amount of time should be foreseen to complete this important stage. Even a method working well under given conditions may require considerable adjustments when applied to real forensic cases. This task should by no means be underestimated.

Inter-laboratories expansion and validation

Ideally, once the method has been established and validated, it can be published in sufficient detail to be implemented in other forensic laboratories. The laboratories which have developed a method could offer consulting and access to their library; access and maintenance of the library will have to be discussed. If several laboratories are actually involved in the development process, such decision can be anticipated. A database can be maintained by a given laboratory which gets samples, analyses and introduces samples into the database, or such a database can be maintained and accessed jointly by several laboratories, which all introduce new samples in a predefined, standardised way. Factors such as money, time, staff, materials, logistics and communications are decisive, and a considerable number of problems are expected to arise in this context. Many laboratories in fact do not have access to the necessary resources, and will therefore not have the means to realistically develop such methods from zero. On a European level, the Questioned Documents European Working Group (QDEWG) of the European Network of Forensic Science Institutes (ENFSI) is now willing and has actually started to discuss the development of dating methods. In addition, a group named International Collaboration on Ink Dating (In C ID) has been created in April 2005. A viable solution would involve the interested institutes to partake in the effort, and divide the tasks within the potential *dating working groups* or *projects*:

- hypotheses definition
- sample acquisition and analysis (composition)
- database construction and maintenance
- determination of aging factors to be studied
- developments of corresponding dating methods
- evaluation of the factors influencing the aging processes
- statistical data analysis
- determination of threshold values
- intra and inter-laboratories validations.

Alternative proposition

Seeing how laborious the tasks of developing dynamic dating methods are, the question arises if the effort invested will be worth in comparison to obtained results? An alternative would be the development of a static dating method through the setting of a “tagging” program in collaboration with the ink industry. The main requests would be collaboration with the manufacturers, price and tracking of measurable tags. The US Service “tagging”-program, which has been restarted in 2000 [LaPorte et al., 2004], used fluorescent markers, which could be detected by HP-TLC. The original program had to stop, because the database recording the tags introduction on the ink market was flawed. This lead to some tags being used twice for different years. Keeping track of the introduced tags in the long term is essential to insure the truthfulness of the dating method. An additional consideration is the increase of the price of ballpoint pens due to these tags, which would likely have to be invested by federal, national or forensic institutions.

5.5 Court and scientific requirements for standards of reliability

On a daily basis the court is confronted to trials in which technical and scientific aspects play a major role. Until recently in the United States, the admissions of an expertise or evidence were evaluated principally according to *Frye*’s standard (Frye v. United States, 54 App. D.C. 46, 293 F. 1013, 1923), which has been formulated in 1923. A scientific expertise was accepted if the validity of the scientific processes was generally accepted among the pertinent scientific community. Later it has been replaced by the Federal Rules of Evidence (FRE) in 1975, which gave the responsibility of evaluating the validity of a scientific expertise to the judge [Kaufmann, 2000]. The debates were strongly raised again in 1993 with the new reliability standards enunciated by the United States Supreme Court in “Daubert v. Merrell Dow Pharmaceuticals, Inc” (509 U.S. 579-601, 1993). The Supreme Court reiterated the necessity of

the competence and qualification of the expert and states that the methods and procedures should be reliable according to the following *Daubert* criteria [Goodstein, 2000]:

- verification of the hypotheses,
- publications,
- known error levels,
- general admission.

Daubert states that the evidence reliability should be decided based solely on principles and methodology, not on the conclusions of the expertise. However, contradictory decisions among trial courts still happen, because judges and jurors have a great deal of difficulty to understand scientific testimony and distinguish the demarcation of science from pseudoscience [Saks, 2000].

Expert testimony based on the “percent dye extraction” dating method raised high controversy and were refused in court on at least two occasions (Regina v. Michael Gurmann in Ontario, Canada, 1993; Learning Curve Toys, L.P. v. Playwood Toys, Inc. 2000 U.S. Dist. Lexis 5130). Today it is generally admitted that these methods are not reliable [Hicks, 1993; Jahns, 2004; Starrs, 2000] and interest has shifted to alternative methods essentially based on the “percent solvent extraction” technique. Even fewer publications exist on the subject and general acceptability does not exist yet among the questioned document examiners.

On a scientific level, Horwitz [1982] published results on interlaboratory validation studies and summarized the important aspects of reliability for an analytical method as following:

- reproducibility (between-laboratory precision),
- repeatability (within-laboratory precision),
- systematic error or bias (accuracy),
- specificity and limit of reliable measurements.

The percentage of outliers, false positives and false negative are also potentially useful suitability index. The smallest amount (or concentration) of material that can be measured with a stated degree of confidence is of great

interest for the validation of method based on solvent quantification. It was effectively established in this work that 53% of the ink initial contents are solvents, which represent approximately 3.5 μg for a 1 cm ink entry on paper. These quantities diminish very quickly below 0.1 μg after a few days and below 0.001 μg after a year. The "Horwitz Trumpet" curve relates the inter-laboratories coefficient (CV) of variation found during proficiency testing according to the concentration levels to be analysed. For a given concentration c = 1 ppm (10^{-6}), Horwitz observed that the CV reaches 16 %. When the concentration decreases, the CV increase according to the following equation [Hall and Selinger, 1985]:

$$CV(c) = c^{-0.15}/50 \qquad \text{Eq. (5.5)}$$

With the extraction procedure used in this work (1 cm ink dissolved in 10 μl extraction solvent), the actual concentrations to be quantified ranged from 0.01% to 0.1 ppm (1 μg - 1 ng) (see fig. 5.11).

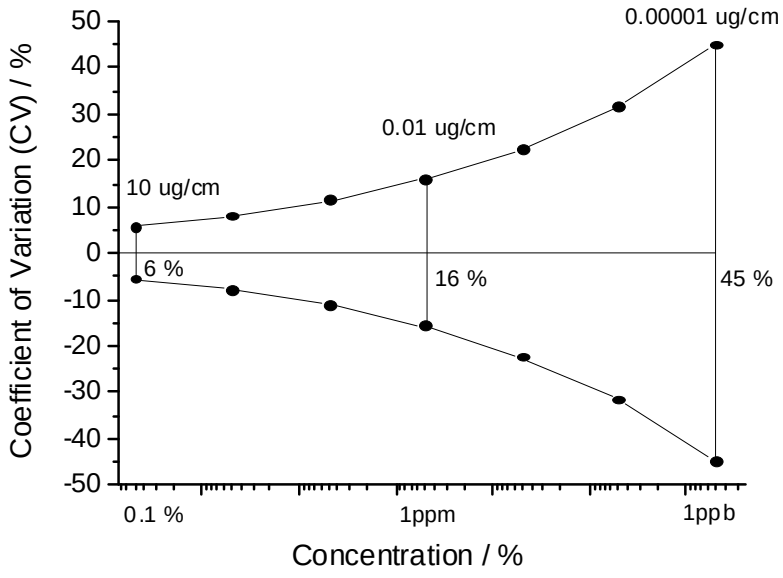


Figure 5.11 Theoretical "Horwitz-Trumpet" for the quantitative analysis of ballpoint solvents by GC/MS calculated with the equation given by Hall and Selinger [1989]. For a concentration of 1 ppm (corresponding to 0.01 μg solvent in 1 cm ink entry), the coefficient of variation (CV) reaches 16%.

Therefore, quantification of ballpoint solvent in ink would be reliable only for fresh inks younger than a few years. For long time span, the obtained difference between the compared quantities (Δc) must be larger than the measurements errors. The nature of the sample yields therefore to essential limitations regarding the establishment of a dating method.

According to the court standards and scientific criteria exposed above, no ink dating method fulfills the requirement for regulatory use in expert testimony yet. Future works should therefore focus not only on scientific standards, but also on the law objectives.

Die Zeit kam so geräuschlos und entfernte sich, ohne dass man es merkte.

Time came on silent wings, and left unnoticed.

Robert Walser

6 CONCLUSION

Since its beginnings, the field of forensic questioned documents has been concerned with the dating of inks. Ink aging processes follow complex paths and disagreements about the feasibility of current methods have been voiced worldwide among the scientific community. This controversy has been the starting point of the present work. Its aim was that of actually studying the aging processes of the dyes and the solvents found in ballpoint pens by modern mass spectrometric methods in order to evaluate the potentiality and limitations of dynamic dating methods.

Laser Desorption Ionisation, Electrospray Ionisation and Gas Chromatography Mass Spectrometry proved to be more adequate and powerful tools than standard methods used today in the analysis of ballpoint dyes and solvents. These techniques yield a sensitive, reproducible, discriminating, rapid and easy analysis with minimal sample preparation. They provide useful information about the compounds found in inks and may be of use in the establishment of an ink database that is essential for static and dynamic dating methods.

Scanning Microprobe LDI-MS, a new non destructive method, has been proposed for determining the crossing sequence of lines on paper, and has given encouraging preliminary results in instances when the ink constituting the lines were not entirely mixed at the intersection.

Many factors influencing the fading of dyes and the drying of solvents over time have been identified, and to some extent measured and evaluated in standard conditions. Variations in ballpoint pen type (ink composition and concentration) and storage conditions (light, temperature, humidity, air flow, paper type) lead to very complex processes resulting in different aging states for an identical point in time. Also, contamination through migration of solvents from one sheet of paper to another was quantified, and should not be

underestimated. Without precise knowledge of these variables and of the extent of their influence, it will not possible to deliver a scientific result for the age of an ink entry. Any established dating method based on the fading of dyes or the drying of solvents has to be checked for reliability through indications of reproducibility, by valid answers in blind testing and by measuring errors that are lower than predictable variations.

On the other hand, the question about the age of an ink entry lies more on the inference of sources rather than the technological or analytical aspects. One can therefore argue that an unequivocal conclusion about the age of an entry will ever be possible in practical forensic cases, as the nature of the samples (small amounts and complex ink-substrate matrix) and the influence of the storage conditions (stack of paper in a file or plastic folder) may actually yield larger differences in the dyes and solvents fractions of the ink entry than the ones provoked by time. The measurement however precise would give no indication about the true age of the ink.

Tempus veritas

Seneca

7 OUTLOOK

The establishment of a functional ink database is an essential prerequisite for dating ink. Static aging criteria are based essentially on an extended knowledge of past and present ink and paper market innovations. Since ink composition is a major factor of influence for the fading of dyes and the drying of solvents, a detailed database would have to include data about corresponding variations of the aging processes. Individual characteristics of ink that remain unchanged with time should be added to help avoid false negative and false positive results in the identification process. Natural isotopes and inorganic trace elements analysed by ICP-MS and IRMS could be a solution if results prove to be reproducible. The most reliable way would be to introduce every year a different measurable tag (e.g. fluorescent marker) into the ink.

For more detailed studies of the fading of dyes, fragmentation patterns provided by ESI-MS^n represent a very promising approach, while thermodesorption with cryo-focusing GC/MS as a method to study the solvent drying processes may prove to be more sensitive and less destructive than liquid extraction of solvents, as well as mass independent.

The influence of the support of the ink on the drying processes should be deeply studied. The adsorptivity, porosity, roughness, pH and coating of the different papers, as well as their fibres and surfaces varnishes have to be evaluated to understand the extent of their influence on the aging behaviour of ballpoint solvents.

The potential of Scanning Mircroprobe LDI-MS for the determination of crossing sequences of lines should be investigated further.

Another significant, but mainly unexplored aging process is the polymerisation of resins contained in small quantities in ballpoint inks. Graphite assisted LDI-

MS has been found to be a powerful tool in investigations of such additives in artworks varnishes [Dietemann, 2001]. The techniques of ESI-MS and MALDI-MS have given promising results on standard resin products used in ballpoint ink formulations [Kirsch et al., 2003a; b].

In future developments of dating methods, the focus should be on the determination of time thresholds that are valid for any given set of conditions (ink, paper, storage) and on interlaboratory validation of the methods by extensive blind testing.

The time is always right to do what is right

Martin Luther King

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Don’t say you don’t have time. You have exactly the same number of hours per day that where given to Helen Keller, Pasteur, Michaelangelo, Mother Theresa, Leonardo da Vinci, Thomas Jefferson and Albert Einstein.

H. J. Brown, Jr

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