

Laboratory Experiment

Identification of paper by stationary phase performance

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ABSTRACT

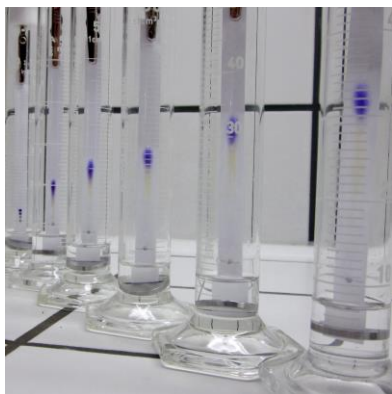
Paper is an extraordinary example of a composite engineering material with practical use in a huge variety of applications. Since its invention in China there have been many alterations to manufacturing techniques, component formulation and surface finishing, but the essential characteristics of the product have not changed greatly.

The objective of this experiment is to demonstrate that the chromatographic performance of paper as a stationary phase may be used to distinguish between externally-similar materials. The procedure developed is so simple that even students with little exposure to practical chemistry and limited chemical knowledge can understand the essential content.

Because of the ubiquitous nature of paper, it is often recovered from crime scenes as evidence for analysis. However, the variety of sources and the mechanical or chemical properties often make demonstration of unequivocal correlation between “questioned” and “known” or “reference” samples a complex task. Introducing the practical session as a crime scene investigation, and presenting the laboratory exercise as an accessible forensic technique for paper identification, adds an exciting dimension to the practical experience for young people.

The authors’ application of this strategy has been successfully extended as it has become evident that the experiment motivates a surprisingly wide range of audiences.

ABSTRACT GRAPHIC



KEYWORDS

High School, First-year undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-on learning, Forensic Chemistry

INTRODUCTION

Paper, to the great majority of the population, is a thin, flexible, composite material used for transmitting printed and hand-written information. The invention of this remarkable material is generally accredited to Cai Lun an officer of the Chinese Imperial Court during the Han Dynasty (202 BC-220 DC).^{1,2} At this time compressed dry fiber mats were used as protective padding, wrapping/writing material, containers (tea-bags) and one of the earliest forms of non-metallic currency. This useful and versatile product rapidly spread to neighboring countries, and by the 13th century to Europe.

As the importance of paper as a commodity increased, manufacturers invested in more efficient production, improved the technical characteristics and reduced costs to increase profits. Through the 19th century wood-chip³⁻⁶ overtook cotton-based rag as the source of the cellulose fiber. Cellulose fibers are extracted from wood using a combination of mechanical, chemical and/or thermal processes. Some of the lignin, and most of the soluble non-polymeric components, are eliminated by copious washing. This simple treatment is appropriate for low-cost papers and newsprint, however residual lignin imparts brittleness and a tendency to discolor after a relatively short period of time.

During the industrial revolution (1750-1850) demand for paper increased substantially and created a need for large-scale production and an improvement in quality. Henry Fourdrinier developed and patented (1801) a

mechanized heating and rolling process to transform high volumes of wood-pulp mash into a surface-finished roll of paper at low cost.

The two principal industrial processes that provided woodpulp were the “sulfite” and kraft processes. The former, originally developed in 1870, has been improved through application of modern manufacturing methods, but the principal stages still use sulfites or bisulfites to sulfonate pulp in high-pressure digesters². This acid process provides a high pulp yield, but the paper lacks mechanical strength. In addition, the risk of serious sulfite water pollution requires effluent treatment, thus increasing production costs.

The kraft process dates from about 1880^{3,5,7} and uses aqueous sodium hydroxide and sodium hydrogen sulphide during heat-assisted digestion. This method lessens damage to carbohydrate fibers and yields a very strong (hence *kraft*, German for strong) high quality paper with excellent storage characteristics.

Further attention to paper chemistry has been motivated by the need to preserve or restore historical documents, and the acid-catalyzed degradation of cellulose has long since been identified as a major problem. Many manufacturers^{7,8} have implemented alkaline production and finishing processes to yield “acid-free” paper with a life expectancy of more than 500 years. After centuries of improvements to production and formulations, and in spite of the environmentally and economically-driven shift towards electronic storage in a paper-free society, printed documents will probably share space with digital records for many decades to come.

FORENSIC ANALYSIS OF PAPER

Paper is a complex material incorporating a wide variety of chemical components.⁷⁻¹¹ On one hand, this presents an analytical challenge of significant complexity. On the other hand, it presents an opportunity, in the sense that papers are identifiable by the chemical signature of the manufacturer’s formulation of optical brighteners, pigments, retention agents, sizing, wet-strength additives, binders and fillers. The identification of the source of a paper fragment may be accomplished through evaluation of: i) physical characteristics (color, dimensions, density, opacity and fluorescence); ii) markings and embossings; iii) component fiber structure; iv)

chemical ingredients in paper conditioning additives; v) trace element content. The elements present in samples of paper can be conveniently identified by neutron activation, X-ray fluorescence and electronic microscopy.¹² In many of these techniques the destruction of a part of the sample recovered from the crime scene as evidence is unavoidable. The need to minimize this consumption of "questioned sample", and perform a complete analysis is an additional challenge for the forensic examiner.

Forensic analysis of paper involves both direct comparison of so-called "questioned" and "reference" paper fragments and searching for matches in specialist databases. The strategies listed above provide many points of comparison, however few laboratories have such a wide range of techniques available. This conclusion led Zideman¹² to develop a simple test that could be applied in virtually any laboratory. In traditional paper chromatography, an unknown mixture of compounds is eluted with a suitable mobile phase and retention factors (R_f) are evaluated. The strategy proposed by Zideman involves application of a mixture of *known* components (referred to herein as "standard sample") to questioned and reference papers followed by elution with a suitable mobile phase. As chromatographic behavior depends on interactions between components of the standard sample and the mobile and stationary phases, the experimental values of R_f provide a simple and sensitive basis for comparing papers. Zideman also noted that test paper sheets cut in perpendicular directions, one in the direction of transport through the paper-making mill (machine direction, MD) and the other at ninety degrees to MD (cross machine direction, CM), often show different chromatographic performance. This difference is caused by partial alignment of cellulose fibers during rolling/pressing. Each single sheet of paper can therefore provide a unique multi-point characterization based on stationary phase performance.

The objectives of this study were to optimize conditions, to confirm the reproducibility of the techniques discussed by Zideman, and to construct a forensic-style laboratory activity suitable for secondary school and first-year university level students.

OPTIMIZED EXPERIMENTAL PROCEDURE

The principal interest in this experiment is to characterize paper as a stationary phase. The preliminary activities in development of an experimental procedure focussed on optimizing the formulation of a “standard sample” with components that become well-separated on a range of “questioned” papers. A satisfactory formulation must have components that are readily visualized using conditions reproducible in school or college teaching laboratories. In addition to this constraint, the mobile phase should be easily prepared, of low toxicity and relatively inexpensive.

The equipment design uses laboratory material that is readily available and the procedure is within the grasp of all science students. Paper sheets are cut into strips, marked and spotted with the standard solution and supported by a clip inside the cap of a 50 mL measuring cylinder (Figure 1). The distance between the spot and the height of the mobile phase in the elution chamber, saturation of the atmosphere of the elution chamber, spot dimension, and duration of the elution process are rigorously controlled to achieve reproducible results.

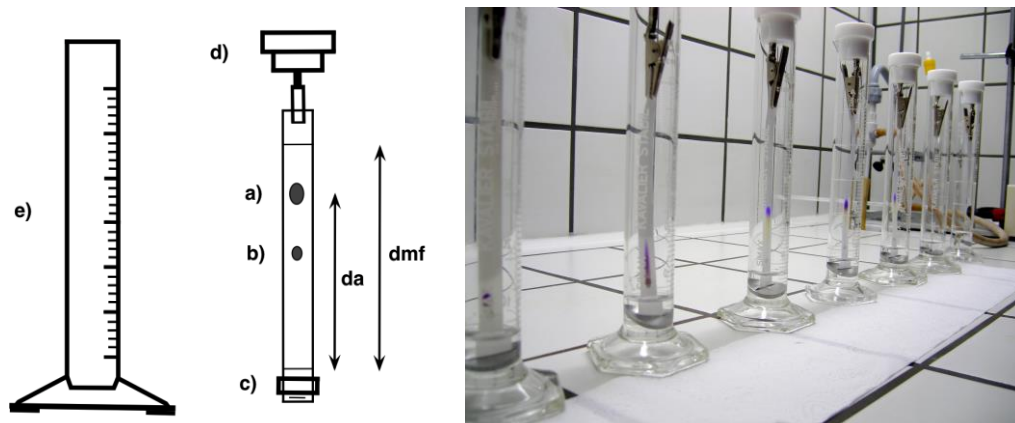


Figure 1. Schematic view of chromatographic chamber and experiment in progress. In the diagram **a)** and **b)** are components of mixture, **c)** disk weight, **d)** teflon cap, **e)** measuring cylinder, **da** is the distance covered by component **a)** and **dmf** is distance covered by the mobile phase front.

Optimization of the operational parameters of this experiment took place in various stages. Using the conditions described by Ziderman as a starting point, a series of indicators and dyes including bromothymol blue, cresol red, eriochrome black T, fuchsine, gentian violet, malachite green, methylene blue, rhodamine B and methyl orange were prepared as 0.2 wt%. methanolic

solutions. Each solution was applied to strips of medium-weight office paper and eluted with methanol, ethanol, propanol, butanol and pentanol as mobile phases. Most of the components used in exploratory experiments were eliminated due to poor visibility or an unsuitable R_f range. The efficiency of chromatographic separation was clearly influenced by the volume of sample solution used. An excessive load of dye/indicator caused “streaking” or “tailing” effects with a visible trail left by the sample as it eluted along the stationary phase.

As a result of this preliminary characterization, the number of candidates included in the standard sample was reduced to four substances, and further reduced in trials of pairs of indicators to two: cresol red and gentian violet. The cresol red component produced a less visible yellow spot on the paper after elution and therefore the concentration of this component was increased. Gentian violet dye eluted as a blue spot. An optimized standard sample has a composition of 0.4 and 0.2 wt% cresol red and gentian violet, respectively.

Chromatographic separation of components of a mixture occurs when the individual components suffer interactions of sufficiently different intensities with the mobile and stationary phases. The interactions that are established are often complex and may be substantially altered by small changes in experimental conditions. In this laboratory exercise the chemicals applied as surface coating, and the cellulose fibers of paper, provide a sufficiently different interaction that results in the separation of the standard sample components to a different degree. In order to optimize the conditions for component separation, the standard sample was subjected to studies with methanol, ethanol, propanol, butanol and pentanol mobile phases and the results are illustrated in Figure 2. When different alcohols are used as the mobile phase in comparative experiments with a specific paper, the difference in the interactions between the alcohol and the stationary phase cause higher alcohols to diffuse more slowly through the stationary phase (Figure 2). This may be understood in terms of a more intense interaction between the chain segments of the higher alcohols and the stationary phase.

Preliminary experiments also confirmed that the resolution of standard sample components varied with ethanol being incomplete, propanol being almost complete, butanol being complete and pentanol being complete but with

tailing (Figure 3). Given the time necessary for sample elution, and the resolving power of each mobile phase, butanol represents a good compromise for most commercial papers.

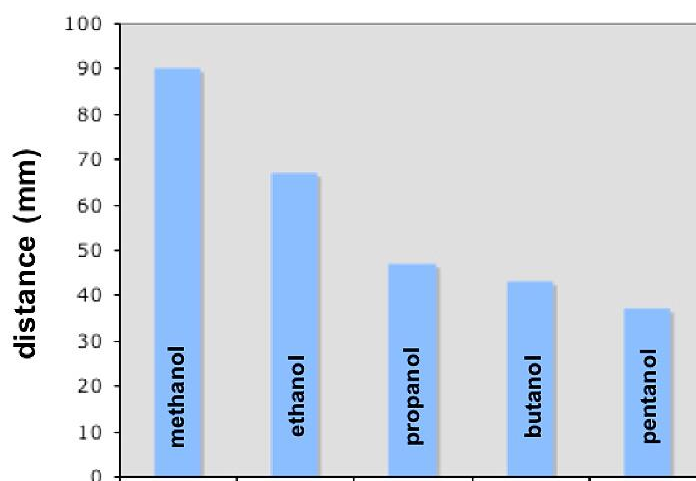


Figure 2. Distance covered by different mobile phases in 40 minutes





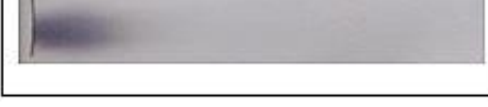
	<u>pentanol, 120 minutes elution</u>
	<u>butanol, 90 minutes elution</u>
	<u>propanol, 90 minutes elution</u>
	<u>ethanol, 60 minutes elution</u>
	<u>methanol, 40 minutes elution</u>

Figure 3. Resolution of the two dyes that make up the standard sample using different mobile phases. Pencil outline added to aid localization of spot.

Both the distance covered by the mobile phase during a fixed time interval and the time taken to reach a fixed reference point were assessed as criteria for marking the end of the chromatographic experiment. From a practical viewpoint, it is easier to reproduce the measurement of distance after a fixed time.

More than twenty commercial papers, with different densities, white color shades, manufacturing processes, pulp feedstocks (recycled or hybrid

recycled/virgin) and application-optimized formulations (laser/inkjet/copy) were characterized. Although some showed similar behavior, it was easy to select six papers with sufficiently different characteristics to serve as the basis for student experiments. The papers shown in Figure 4 are of similar weight and color but with different application optimizations and fiber compositions. A preliminary study of locally available printing and office papers may be expected to provide suitable material for study.

FORENSIC LABORATORY EXERCISE

In a typical student laboratory experiment, six reference paper samples and a “questioned” paper were provided as stationary phases in evidence collection envelopes. Students cut strips, marked a starting reference line and applied the standard sample to each paper. The paper strips were placed in 50 mL measuring cylinders with butanol as mobile phase, loosely stoppered to preserve a constant atmosphere, and eluted for 90 minutes. The strips were removed from the tanks, the position of the mobile phase front quickly identified and components of the sample located and marked (Figure 4).



Figure 4. First six commercial papers (counting from left) with "questioned" sample (far right) using butanol as mobile phase and 90 minute elution. Pencil outline added to aid localization of spot.

The results illustrated in this figure are from one student group, however reproducibility between different groups is typically about ± 1 mm, or approximately 2-3%.

Using this procedure the students obtain three points of comparison from each paper strip: the distance run by the mobile phase front and the position of two spots. Some groups cut paper strips in transversal orientation and although the distance covered by the mobile phase front during the same time interval was shorter, R_f values were comparable (Figure 5).

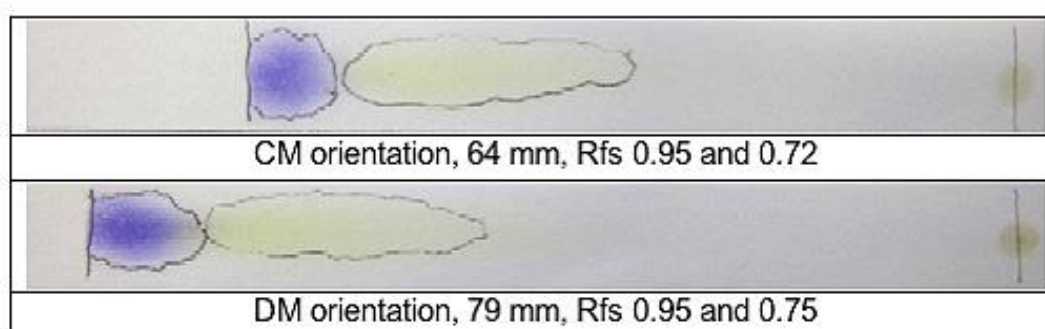


Figure 5. Comparison of commercial paper behavior in DM and CM orientation (butanol mobile phase and 90 minute elution). Pencil outline added to aid localization of spot.

HAZARDS

Appropriate safety equipment should be used by the students at all times (laboratory coat, safety goggles and gloves). The mobile phase (n-butanol) and standard sample solvent (methanol) are flammable but represent a relatively low risk.^{13,14} Although very low quantities of these alcohols evaporate during the experiment, it may be considered prudent to conduct this experiment in a fume hood.

RESULTS AND DISCUSSION

Results obtained by almost fifty groups of students since the introduction of the experiment have confirmed a close to 100% success rate in identifying the “questioned” office paper. The reproducibility of measurement is generally better than ± 1 mm, as illustrated in Figure 6. This laboratory exercise is experimentally robust but care in spot application is required to retain compact stains after elution. More precise location of each stain can be obtained by scanning the paper and digitally evaluating spot color intensity.

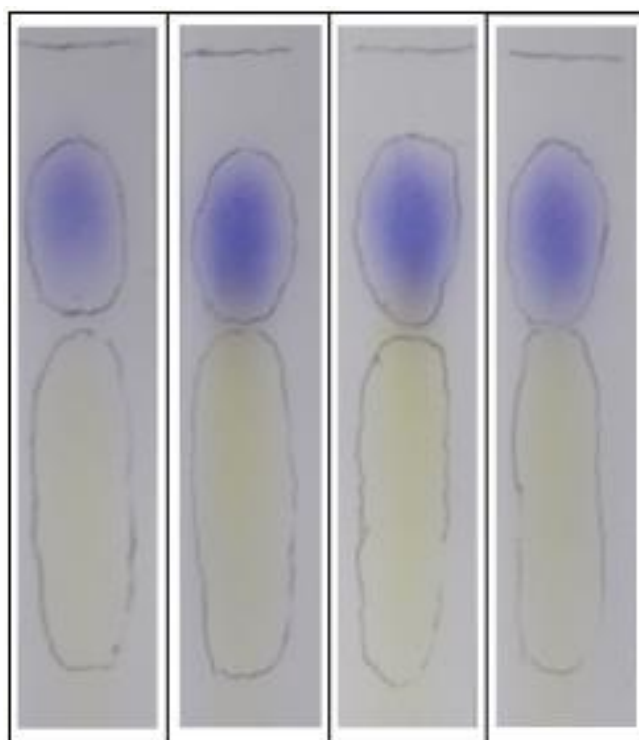


Figure 6. Comparison of four student replications of the application of standard sample to the same commercial paper. Pencil outline added to aid localization of spot.

The content of the practical class provides a natural opportunity for students to question what interactions cause separation of the sample components. The structures of cresol red and gentian violet are chemically very different and are included for reference in the supplementary material. The aromatic rings of cresol red adopt a bulky skewed orientation, however the pendant hydroxyl groups can form hydrogen bonds with hydroxyl groups on the linked D-glucose units of the cellulose fibers at the paper surface. The gentian violet structure contains three aromatic rings and is probably a more planar molecule that, given the nature of the NH_2 groups, offers a weaker interaction with the surface groups on the stationary phase. This is consistent with the stronger retention of cresol red relative to gentian violet. With less polar butanol or pentanol mobile phases, the difference between the behavior of the two components is increased and better chromatographic separation is achieved.

This experiment can be readily adapted for a younger audience. Instead of applying very small volumes of the standard sample, a series of 15 black ball-

point pens were characterized with different mobile phases on office papers. Four of these pens contained inks with two or three components that fitted our previously described criteria. In this simplified experiment, the “standard sample” was applied using the selected pen to mark a spot on the paper. To avoid excessive ink load students drew a 3mm diameter circle using a spiral movement, passing the pen point only once over the paper surface. Further experimental details for students have been included in the supplementary material as support for practical aspects of this experiment and additional content including “in-laboratory questions” and “post-laboratory projects” is also available.

CONCLUSION

Our experience with the approach described in this paper has been very favorable and we have found that students show markedly more interest in investigating characteristics of materials within a CSI scenario. Increased motivation is also reflected in the attention that students invest in precise execution of the experimental procedure, a more participative discussion while students prepare preliminary responses to in-lab questions, greater investment in post-laboratory reports and even suggestions for improvements to the method. The explanation of chromatographic separation at the level at which this experiment is proposed is quite demanding. The students have little experience or specific knowledge of the nature or relative intensity of interactions between chemical components and the mobile and stationary phases. This simple experiment in paper chromatography provides an early opportunity for a preliminary dialogue. Once the teacher has captured the attention of the student with an appropriate learning scenario, the student often surpasses the teacher’s expectations and embarks on the search for evidence that leads to a most satisfactory academic conclusion.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the support provided through laboratory facilities in both host institutions and additional equipment and financial provision through the Centro de Química (FEDER-COMPETE/QREN/EU and PEst C/QUI/U1686/2013, Fundação para a Ciência e Tecnologia).

ASSOCIATED CONTENT

Supporting Information

Notes for instructors, step-by-step procedures for students, procedure for simplified experiment. This material is available via the internet at <http://pubs.acs.org>.

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