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# On the Relative Aging of Ink—The Solvent Extraction Technique

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**ABSTRACT:** The relative aging of ink is the comparison of inks of the same formula and on the same paper to ascertain the order in which they were written. The solvent extraction technique determines how efficiently an ink is extracted into a solvent. This efficiency involves both the rate and extent of extraction and is dependent on the time the ink was placed on the document. Two procedures are presented for obtaining the extraction rates: the $R$ ratio and the $L$th extraction time procedure. The third procedure presented, the sequential solvent extraction procedure, measures the extent of extraction as percent extraction. This procedure can be extended to provide extraction rates. Two methods are presented for obtaining either the extent or rate of extraction: a thin-layer chromatographic (TLC) method and a spectrophotometric method. A suggestion is made to obtain the extent of extraction using a modified TLC method.

**KEYWORDS:** questioned documents, inks, chromatographic analysis, relative aging, storage conditions, paper aging, solvent extraction, solubility, thin-layer chromatography, densitometry, high performance liquid chromatography, ultraviolet-visible and fluorescence spectrophotometry

There are two different approaches taken for dating inks on manuscripts. One determines static (compositional) characteristics of the inks and the other determines dynamic (drying) characteristics. In the former approach, a compositional profile is obtained from a questioned ink and this is compared with those from inks in a standard reference file. When a match is found, the inference is that the questioned ink cannot be any older than the date that the matching standard was first produced. The method of this static approach and its limitations have been presented in previous works [*1-5*].

This paper deals with the dynamic characteristics of ink aging. There have been three independent approaches to determining these characteristics in ballpoint inks. One approach follows the disappearance of the ink's volatile components as the ink ages [*6*], the second follows the changes in certain of the ink's infrared absorption peaks [*7,8*],[3] and the third approach follows the decrease of the ink's extraction efficiency into a solvent [*9-11*].

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This third approach is the subject of this paper and it is hoped that it will lay groundwork for further research.

The dynamic process of ink aging on paper not only involves the evaporation of the volatile components, but possibly also oxidation, polymerization, ink-paper interactions, or a combination of these. Whatever the mechanism may be, this process is measurable; it monotonically slows down and ceases after a period of time. Dating an ink by following its aging on paper can be done by comparing this aging with the aging of inks of known age provided they are of the same formula and are on the same document as the ink in question. This is referred to as relative aging determination.

## Some Principles of Relative Aging

Before proceeding with the extraction efficiency techniques, it is necessary to first present principles which apply to any relative aging technique [*9,10*].

### *Comparison of Inks on Paper*

As mentioned above, relative aging can be of value when at least two conditions are met:

1. The inks being compared are of the same formula. This is done by comparing the compositional profiles using, for example, optical and multiple thin-layer chromatographic (TLC) methods [*1-5*]. The ink library approach used by the Bureau of Alcohol, Tobacco and Firearms (ATF) has shown that matching such profiles indicates that the inks are of the same formula. It thus appears that matching profiles assures the same formula of ink; however, it is advisable to identify the specific formula, and thus the manufacturer, to be sure of its uniqueness. Since the manufacturing of ballpoint ink involves extremely high quality assurance, each batch made should be identical in its drying characteristics. Also it is safe to assume that any ink drying within a pen cartridge is negligible compared to the ink drying when exposed on paper.
2. These inks appear on the same paper. Here we assume that whatever exposure the paper has had over time it has been uniform on all of the paper. Since paper is hygroscopic, its water content can vary from day to day. If the measurement of ink aging is dependent on this, then it is advisable to perform ink aging comparisons during the same day while temperature and humidity conditions are the same—or during equivalent conditions.

### *Effects of Storage Conditions*

If inks of the same formula are on separate papers but of the same composition and age, then knowledge of their storage conditions is necessary to make valid comparisons. For example, suppose two inks are on separate documents (same composition and age) and one ink has a known date of writing ($K$) while the other does not ($Q$). If relative aging shows $Q$ to be younger than $K$, then this can be valid if the storage conditions of $K$ are the same as those of $Q$ or less age inducing. Similarly, if relative aging shows $Q$ to be older than $K$, this can be valid only if the storage conditions of $K$ are the same as those of $Q$ or more age inducing. For inks on the same paper, *the effects of storage conditions are incorporated in their aging.*

### *Effects of Paper Aging*

The aging of ink can be determined in at least two of ways: (1) determining, at one time, the age of an ink written at several different times, and (2) determining, at several different times, the age of an ink written at one time. *Each of these incorporates the effect of paper aging into the aging of its inks,* and thus paper aging is not a concern in the comparison of

inks in either of these ways. However, the aging results obtained from one are not identical to those obtained from the other unless paper aging is not a factor.

If it is necessary, a suggested procedure for determining the effect of paper aging on ink aging is to place a line ink entry periodically on the paper; every time a new line is placed, its age is determined along with the age of each previous line. In the case where, for example, 5 lines are entered, there are $5 + 4 + 3 + 2 + 1 = 15$ age determinations. The 5 samplings of the first line are of Type (1) and the last samplings of each line are of Type (2). The Type (1) way of determining ink age is more like the actual cases encountered and comparing this with the Type (2) way becomes important in the following special cases.

*Case 1*—Determining the age of a single questioned ink entry on a document is sometimes possible. A sample of the ink is needed to place it on the document at several different times over a period equivalent to, at most, the alleged age of the single questioned ink (if known) and, at least, the actual date of the single questioned entry (if known). These "planted" ink entries are left to age under controlled laboratory conditions. For making valid comparisons between the single questioned entry and the "planted" entries, these conditions should be the same as, or less age inducing than, the conditions under which the questioned ink had been previously stored. Since this method of comparing ink aging is subject to the effects of paper aging, one has to determine if ink ages in the same way when placed on the same paper at two different times.

*Case 2*—The case where inks of the same formula are on separate papers of different composition (or of the same composition but of different age) has to be treated with extreme caution, if treated at all. Not only is the knowledge of their relative storage conditions necessary, as discussed above, but one needs to determine *how ink ages on the different papers.* One can attempt an "allowed controlled aging" procedure using "planted" ink entries, like the one discussed above, on both papers to see, for example, if the ink drying characteristics are the same, or proportional, for both papers. In this case, a simple proportionality constant relates the results from one paper with those from the other. Since this comparison carries the possible effect of paper aging, one also has to determine how ink ages when placed on each paper at different times.

In either of these cases, if it can be shown that ink ages faster than the paper on which it appears, then there should not be any paper aging effect. We speculate that this may be what happens in most cases though testing is still necessary.

### *Mass Invariant Measurements*

In comparing one ink with another, the parameter being compared must be invariant of mass. That is, this parameter must be independent of the amount of ink sampled since there is no practical way to assure that the same amount of ink is being sampled all the time. In every relative aging determination, the parameter involves particular ratios of measurements to meet this requirement. Beer's law, that is, the measured parameter (for example, optical color density) of a substance in a given matrix (for example, in a solution) is proportional to its concentration, is the basic reason these ratios are mass invariant. Despite this protection, attempts should be made to sample *similar amounts of ink in quantity and line quality* to reduce any possible error.

### *Aging Process and Accuracy of Age Determination*

A question often asked about relative aging concerns its accuracy. Since the relative aging techniques being studied present ink aging as a monotonic process which slows down and levels off with time, something like an "exponential" behavior, the accuracy decreases dramatically as the process levels off. Thus, it may be impossible to distinguish two inks with a small age difference between them but with a large age difference between the time either

was written and their time of analysis. Also, if two inks being compared show no difference in their relative age, then they are of the same age if they still continue to have measurable aging, that is, their aging has not leveled off yet.

The ink aging process, as mentioned, is a monotonic process which reaches an age beyond which no differences can be detected. Because the process behaves "exponentially," this leveling off age is related to how fast it is reached, that is, the rate of aging which depends on the ink formulation. This is why some similarly stored ink formulas age faster than others and why inks of the same formula are required for comparison.

## General Principles of the Solvent Extraction Technique

The solvent extraction technique [*9,10*] involves the measurement of extraction efficiencies. It is based on the premise that the longer an ink has been on paper, the drier it should become and the harder (less efficiently) it will extract into a solvent. Conversely, the fresher the ink, the easier (more efficiently) it will extract. This extraction efficiency involves both the rate of extraction, that is, how fast the extraction takes place, and the extent of extraction, that is, how much is finally extracted.

### *Extraction Curves*

As an ink extracts, the concentration, and thus the color, of the ink in the extracting solvent increases. The concentration versus the extracting time constitutes an *extraction curve*, $E(T; t)$, where $T$ is the age of the ink on paper and $t$ is the extracting time. This curve increases monotonically and slows down with time leveling off at some value (asymptote), $E(T; \infty)$, the final extent of extraction.

Theoretically, the leveling off time is infinite, as indicated; but in practice there is a finite leveling off time as a result of the finite margin of error associated with the absorbance measurements. In the equations that follow, we shall continue to consider this leveling off time as infinite.

### *Extraction Rate Curves*

The extraction curve can be obtained by measuring the absorbance of the colored solution. This can be at the wavelength where maximum absorbance occurs. If Beer's law is valid (this will be shown to hold), each absorbance value (ordinate) of an extraction curve is proportional to the concentration of the ink which, in turn, is proportional to the amount of ink sampled. Another way of expressing this is that extraction curves of the same ink but corresponding to different amounts sampled are proportional to each other. Consequently, the ratio of any two ordinates of an extraction curve cancels any mass dependency; it depends only on the rate of extraction. A *mass invariant extraction rate curve* can thus be generated from an extraction curve by normalizing it by its asymptote (that is, dividing each of its ordinates—absorbance values—by the asymptote);

$$X(T; t) = \frac{E(T; t)}{E(T; \infty)} \tag{1}$$

In fact, dividing by any absorbance value less than the asymptote yields a "*modified*" *mass invariant extraction rate curve.* For example, if an *incomplete extraction curve* is obtained by stopping at a time $t'$ before extraction ceases, then dividing each absorption of this

curve by the final absorption measured produces a mass invariant extraction rate curve which we refer to as "modified":

$$X'(T; t) = \frac{E(T; t)}{E(T; t')} \tag{2a}$$

where

$$t < t' < \infty \tag{2b}$$

*Rate and Extent of Extraction*

By Eq 1, an extraction curve has two components: (1) the asymptote, $E(T; \infty)$, or extent of extraction which depends only on the amount sampled; and (2) the function $X(T; t)$ which depends only on the rate of extraction;

$$E(T; t) = E(T; \infty)X(T; t) \tag{3a}$$

There is a similar equation involving an incomplete extraction curve,

$$E(T; t) = E(T; t')X'(T; t) \tag{3b}$$

The *sensitivity* of the extraction curve $E(T; t)$ to aging (the derivative with respect to $T$) is the sum of the sensitivity of $E(T; \infty)$ and $X(T; t)$;

$$[\partial E(T; t)/\partial T] = [\partial E(T; \infty)/\partial T]X(T; t) + E(T; \infty)[\partial X(T; t)/\partial T] \tag{4}$$

If ink aging does not involve changes in rate of extraction, then mass invariant rate curves cannot be used for comparing inks of different age. If aging of ink causes more change in the extent rather than in the rate of extraction, then a mass invariant method must be found to measures these extents.

*Sequential Solvent Extraction*

Extents of extraction must be made independent of mass to compare them for relative age determinations. One way of doing this is by the sequential solvent extraction procedure where, after first extracting the ink in a given solvent for a given time $t$ (for example, until no more ink can be removed which we have called $t = \infty$), the ink remaining in the residue from the first extraction is extracted in a second "all-extracting" solvent *until all the remaining ink is removed* (though this may occur within a few minutes in the "all-extracting" solvent, we designate this time by $\bar{t} = \infty$). If the two extracting volumes are the same, then the absorbance of the first extraction divided by the sum of this absorbance and the absorbance of the second extraction becomes the *percent of extraction* of the ink (for a given time $t$ in the first solvent);

$$P(T; t) = \frac{E(T; t)}{E(T; t) + \bar{E}(T; \infty)} \times 100 \tag{5}$$

where $E(T; t)$ is the extent of extraction of the ink in the first solvent after $t$ minutes of extraction and $\bar{E}(T; \infty)$ is the extent of extraction of the *all* the ink remaining on the residue after the first extraction. It is a percent extraction since the denominator represents the *total*

*mass of ink sampled* for analysis. This percent of extraction is clearly independent of the amount sampled and thus represents a *mass invariant extent of extraction.*

To obtain the *percent extraction curve* $P(T; t)$, $t$ varying, one needs to obtain first the extraction curve $E(T; t)$, which we assume has been taken up to the leveling off time $t = \infty$ (in the first solvent), and its corresponding normalized rate curve $X(T; t)$. One then performs the second solvent extraction and computes $P(T; \infty)$. Then,

$$P(T; t) = P(T; \infty)X(T; t) \tag{6a}$$

or, for an incomplete extraction curve,

$$P(T; t) = P(T; t')X'(T; t) \tag{6b}$$

Compare these with Eqs 3a and 3b.

*Extraction of Individual Dye Components*

An extraction curve is the sum of the individual extraction curves for each of the dye components:

$$E(T; t) = \Sigma E_i(T; t) \tag{7}$$

where the sum is over the number of dye components. These single dye extraction curves may differ from each other since the individual dyes may be selectively extracted by the solvent. Clearly, if *separation methods* (for example, thin-layer chromatography [TLC] or high performance liquid chromatography [HPLC]) are incorporated into the absorption measurement, then the individual extraction curves can be addressed. This is treated later when a separation method is suggested.

*Exponential Extraction*

It is of interest to point out that the extraction curve $E(T; t)$ or the corresponding mass invariant extraction rate curve $X(T; t)$ is not necessarily exponential; if it were, one would have a single number, the rate constant, to characterize the curve. Even if the single dye extraction curves are exponential, for example,

$$E_i(T; t) = E_i(T; \infty)X_i(T; t) \tag{8a}$$

where

$$X_i(T; t) = \{1 - \exp[-\beta_i(T)t]\} \tag{8b}$$

$E_i(T; \infty)$ is the asymptote for the individual dye and $\beta_i(T)$ is the individual dye rate constant, the total extraction curve (the sum of exponential curves) is not exponential unless the individual dyes extract at the same rate. However, at the two extreme extraction times, at the beginning and at the leveling off value, the total extraction curve can be shown to be exponential.

## Experimental Procedure

### *Ink Samples*

*Artificially Aged Samples*—Lines of Fisher pressurized black ballpoint ink were placed on Nashua (photocopy) paper and this was placed in an oven set at 100°C. Every 5 min, for at least 1 h, the paper was removed for placing more ink lines on it.

*Naturally Aged Samples*—One of the authors (AAC) used the same Fisher pressurized black ballpoint pen to document telephone conversations on notebook pads. The paper was not Nashua paper. A pad which spanned a period of two-and-a-half (2.5) years was used for following the ink aging.

The Fisher ink used has two fluorescent rhodamine-type dyes which aid in following the extraction efficiency through fluorescence analysis.

### *Methods to Obtain Extraction Curves*

The solvent extraction technique involves the measurement of extraction efficiencies—rate and extents. These result from the extraction curves—the concentration measurements of the extracting inks. Concentration measurements can be obtained using several methods:

(1) ultraviolet-visible (UV-Vis) spectrophotometry using microcells,
(2) TLC using densitometry,
(3) HPLC using a variable wavelength detector, and
(4) fluorescence spectrophotometry.

To obtain an extraction curve, the chromatographic methods (TLC or HPLC) would require successively removing samples from the extracting solution for measurement. Depending on how much ink sample was removed for analysis, this provides only a few points on the extraction curve.

The spectrophotometric methods (UV-Vis or fluorescence) are more amenable to direct extraction measurements and thus can provide a more complete extraction curve.

Of all the methods listed, TLC is the only one that does not reflect the solvents used to extract since densitometry is performed on the dried spot of extracted ink. As we shall see, this makes TLC be one of the more tractable methods.

*A Chromatographic Method*—A method for obtaining points of an extraction curve involves removing aliquots of the extracting solution at different preselected extracting times and measuring their color. This is done indirectly by spotting the aliquot on a TLC plate (such as E. Merck Silica Gel) and taking a densitometric reading (at the maximum absorbing wavelength). In a typical case:

1. Fifteen micro discs or about a 10-mm sliver of ink on paper are removed using a boring device or a scalpel, respectively, and placed in a Kontes 0.3-mL vial with cone shaped interior.
2. Ten microlitres of the extracting solvent is added at time $t = 0$ min using a Eppendorf pipetter.
3. (Dade) pipets, 1 $\mu$L, are used to remove the 1-$\mu$L aliquots of the extracting solution at the different preselected extracting times. As many as four aliquots may be removed for TLC spotting and subsequent densitometric measurements.
4. Gentle stirring with a needle can be made between sample removal.
5. Densitometric measurements are made using the Shimadzu densitometer (dual wavelength TLC scanner) Model CS-930. Reflectance measurements are made by linearly scanning the TLC plate in the single wavelength mode. The wavelength chosen should be around the maximum absorbance of the ink. For blue and black inks a value of 580 nm is adequate.

The successive densitometric readings should be corrected for the successive solvent removal after each sampling. For removing aliquots at four different preselected extracting times, these are:

$$\text{corrected 1st reading} = \text{1st reading} \tag{9a}$$

$$\text{corrected 2nd reading} = (9/10)\ \text{2nd reading} + (1/10)\ \text{1st reading} \tag{9b}$$

$$\text{corrected 3rd reading} = (8/10)\ \text{3rd reading} + (1/10)\ (\text{1st reading} + \text{2nd reading}) \tag{9c}$$

$$\text{corrected 4th reading} = (7/10)\ \text{4th reading} + (1/10)\ (\text{1st reading} + \text{2nd reading} + \text{3rd reading}) \tag{9d}$$

The pattern can be seen if more measurements are done. This can be generalized for different volumes of extracting solvents and aliquots.

A choice for the last extracting time should be close to where the extraction time is almost complete (for example, 30 min), while the first could be within the first 5 min of extraction, the second with the next 5 min, and so on, if enough solution is available for measurement.

*A Spectrophotometric Method* [11]—Fluorescence measurements are extremely sensitive because they measure emitted light relative to a nonemitting (black) background. If a TLC plate of an ink shows that it has a fluorescent component when a "forensically acceptable" sample size of ink is used (for example, seven micro discs or about a 5-mm sliver of ink on paper as mentioned before), then this fluorescence can be observed and measured using a fluorescence spectrophotometer when this same amount of ink is placed in a conventional fluorescence cuvette with as much as 1 mL of solvent. Consequently, monitoring the extraction of fluorescent components becomes tractable because one can work with larger volumes of extracting solvent and there is *no need to successively remove* samples of the extracting solution for measurement.

In the method developed:

1. Place the removed ink samples (between three to nine micro discs) in a dry syringe whose needle's inner diameter is small enough to keep the samples (discs or slivers) from coming through, for example, a 250-$\mu$L Hamilton syringe with removable standard needle, Series 800.
2. The sample cuvette is filled with 1 mL of the extracting solvent and placed in a Perkin-Elmer (Hitachi) Model 650-40 fluorescence spectrophotometer.
3. The excitation and emission wavelengths are set to optimize the fluorescence of the extracting component. For the rhodamine dyes in the Fisher ink, excitation is at 525 nm and emission is at 552 nm.
4. The extracting solvent is drawn into the syringe at time $t = 0$; it is in this syringe where the extraction takes place.
5. A sufficient portion of the extracting solvent is periodically placed back into the cuvette for measuring how much has extracted. This can easily be done every 1/2 min and a rather well-defined extraction curve can be obtained.

This method works well when there is no interference from paper fluorescence. Since the latter fluorescence occurs when UV light is used as an excitation source, the desired ink component should be one that can fluoresce when other exciting sources are used. Fortunately, there is a series of such dyes that are used in the ink industry. These are excited by UV light but more efficiently by particular visible wavelengths. The rhodamine-type dyes, as found on the Fisher ink used, are of this class. Excitation around 525 nm causes fluorescence around 552 nm for one of the dyes. One should also avoid using solvent which exhibit interfering fluorescence.

Extraction curves for inks without fluorescent components are discussed in the next section. This includes the use of fluorescence "absorption" and direct absorbance methods.

*Procedures for Comparing Extraction Rate Curves*

By Eq 1 or 2, extraction rate curves are obtained from the extraction curve by dividing each ordinate by the final ordinate of the extraction curve. These rate curves can be compared by several procedures. We present two.

*The* R *Ratio Procedure*—This procedure involves *comparing ordinates of the rate curves corresponding to a given extraction time.* In the chromatographic method discussed above, dividing the first three corrected readings of Eq 9 by the last one yields three selected ordinates of the extraction rate curve. (If the last extraction time is not where the extraction is almost complete the resulting curve is the "modified" rate curve.) These three ordinates, along with their corresponding preselected extraction time, form three points on the extraction rate curve and these points can be used for comparing extraction rate curves. Since these ordinates are ratios of absorbance measurements at two different extracting times, the procedure is referred to as the *R ratio procedure* [*9*] and the ordinates of the extraction rate curve as *R* ratios.

In theory, only one point is necessary for comparing extraction rate curves, and thus only two absorbance measurements at two preselected extracting times are necessary. This was the original premise of the *R* ratio procedure [*9*], but the problem always remained on the optimum choice of the two extracting times. Consequently, if more points of the extraction rate curves are available for comparison, this problem is minimized.

*The* L*th Extraction Time Procedure*—In this procedure one *compares the extracting times corresponding to a given ordinate of the rate curve.* This is done by measuring the *L*th extraction time ($0 < L < 1$) which is that time at which the extraction is $[L \times 100]\%$ complete. Clearly, one can work directly with the extraction curve to obtain these times.

This procedure is suited for extraction curves that are rather well-defined instead of those with only a few points. Such more complete curves can be obtained using the chromatographic method presented if sufficient sample is afforded.

The fluorescence spectrophotometric method does provide such curves. In this case, dividing each of fluorescence values forming the extraction curve by the last one yields a rather complete extraction rate curve or a "modification" of it. This curve will have more points available for comparison using the *R* ratio procedure—comparing ordinates at given extracting times. In this way the optimum extracting time for comparison, which gives the maximum discrimination and reproducibility, can be readily found.

The optimum *L* can also be obtained when a more complete curve is available. Note that this procedure involves a fixed absorption while the *R* ratio procedure involves a fixed extraction time. These two forms of measurements are illustrated in Fig. 1.

These two ways of comparing extraction rate curves are only two of any number of other ways. For example, taking any fixed line (or graph) that traverses the mass invariant curve provides a point on that curve for comparison.

*The Sequential Solvent Extraction Procedure for Measuring Extents of Extraction*

Measurements in this procedure are taken at two times: when the first extraction is complete or nearly complete and when the second (total) extraction is complete. The following procedure can be used:

1. Extract ink for $t$ min in $v$ $\mu$L of solvent $S$ ($t$ should be long enough to attain complete extraction).
2. Measure the concentration $C$ of this solution extract. Remove all remaining solvent from residue.
3. Add to ink remaining in the residue from the first extraction $\bar{v}$ $\mu$L of solvent $\bar{S}$ and extract for $\bar{t}$ min. ($\bar{S}$ should be strong enough that it does not discriminate different ink ages,

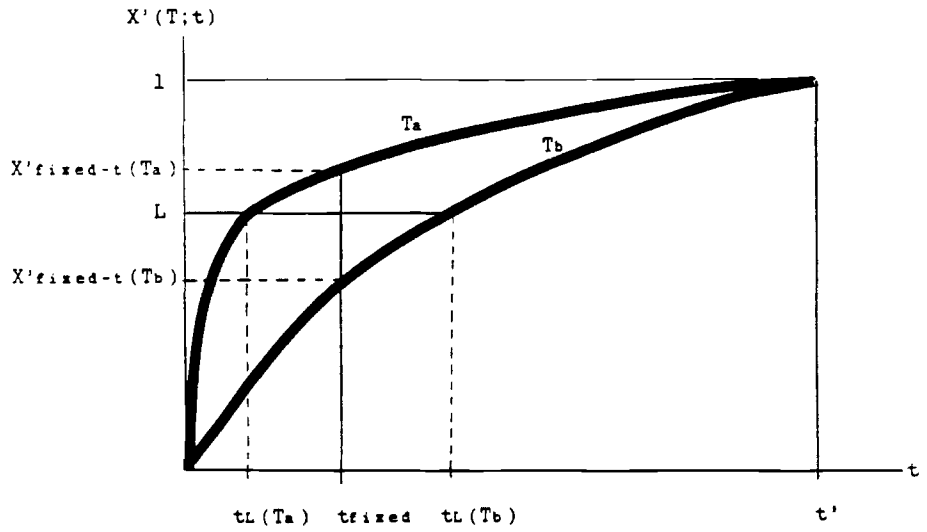


FIG. 1—*The two ways of measuring mass invariant "modified" extraction rates: fixed ordinate (absorption),* L, *and fixed abscissa (extraction time),* $t_{fixed}$. *For curves corresponding to age* $T_a$ *and* $T_b$, *the former yields the comparing parameters* $t_L(T_a)$ *and* $t_L(T_b)$, *respectively, and the latter,* $X'_{fixed-t}(T_a)$ *and* $X'_{fixed-t}(T_b)$, *respectively.* t' *is the time chosen before total extraction occurs.*

that is, it pulls all ink regardless of age. $\bar{t}$ should be long enough to extract all remaining ink.)

4. Measure the concentration $\bar{C}$ of this second (sequential) solution extract.
5. Then, Eq 5 becomes,

$$\text{Percent Extracted in Solvent } S \text{ after } t \text{ min} = P = \frac{vC}{vC + \bar{v}\bar{C}} \times 100 \qquad (10)$$

This procedure is suitable for TLC measurements. For fluorescence measurements, the two measurements can be done in the same cuvette without removing it from the spectrophotometer:

1. The ink samples are placed in the syringe; $v$ mL (for example, 1 mL) of the first extracting solvent $S$ are placed in the cuvette and is drawn into the syringe for the first extraction.
2. After extraction (which may involve stirring by delivering the extract into and redrawing from the cuvette at several predetermined times), the extract is placed back into the cuvette for the first fluorescence measurement $F$.
3. $\bar{v}$ mL (for example, 0.25 mL) of the second extracting solvent $\bar{S}$ are drawn into the syringe for the second extraction.
4. After extraction (with stirring, if necessary, done using a vial), it is then placed in the cuvette with the first extract for second fluorescence measurement $\bar{F}$.
5. Then, Eq 5 becomes,

$$\text{Percent Extracted in Solvent } S \text{ after } t \text{ min} = P = \frac{v}{v + \bar{v}} \times \frac{F}{\bar{F}} \times 100 \qquad (11)$$

By first obtaining an extraction curve, $E(T; t)$, either partially (TLC method) or more completely (fluorescence method), and then performing the sequential extraction, one can

obtain the *percent extraction curve*, $P(T; t)$ (Eq 6). Normalizing this curve by dividing by the last ordinate provides the extraction rate curve. Extraction rates can then be compared using the $R$ ratio procedure when only the partial curve is available or the $L$th extraction time procedure when the more complete curve is obtained.

### *Materials and Equipment Used*

*Sampling Devices*—A syringe needle, 18-gauge, made into a boring device using jeweler's files and having a plunger to remove bored out samples, a scalpel with Size 15 blade, hard clear plastic as the supporting device on which the paper is placed for sampling, and Dumont No. 5 biological dissecting forceps were used.

*Extracting Vessels*—Kontes 0.30-mL vials with cone shaped interiors and a 250-μL Hamilton syringe with removal standard needle, Series 800, or a similar syringe (so long as the ink samples do not escape the cylinder through the needle orifice) were used.

*Solvents*—Methanol (Baker Absolute Photrex—for spectroscopy), benzyl alcohol (ACS grade), toluene (ACS grade), and deionized water were used.

*TLC Materials*—E. Merck Silica Gel 60 TLC plates, 20 by 20 cm, without fluorescent indicator; 10-, 20-, 25-μL multivolume Eppendorf pipetter; and Dade 1- and 5-μL pipets were used.

*Instruments*—Perkin-Elmer (Hitachi) fluorescence spectrophotometer Model 650-40 and Shimadzu densitometer Model CS-930 were used.

### *Experiments*

*Experiment* 1—*Verification of Beer's Law*—As was indicated in the text, Beer's law is the key to obtaining mass invariant measurements. To test Beer's law, the fluorescence method was used to obtain extraction curves up to 15.5 min from three, six, and nine discs of fresh Fisher ink (placed on the paper the day before). The extracting solvent was 25% aqueous methanol.

*Experiment* 2—*Percent Extraction Versus Age Versus Solvent Strength*—The fluorescence method was used to determine the percent extraction of Fisher ink artificially aged up to 165 min. The first extracting solvent consisted of aqueous dilutions of methanol: 75, 50, 25, and 12.5%. The ink was extracted for 10 min in 1 mL of the first solvent. The second extracting solvent was 100% methanol and the residual ink was extracted for 3 min in 0.3 mL of this solvent. Here a 1-$cm^3$ tuberculin syringe, modified to keep ink samples from escaping by using a glass bead in the cylinder, was used as the extracting vessel. Several measurements were repeated.

*Experiment* 3—*Percent Extraction Curve*—The TLC method was used to obtain a percent extraction curve of fresh ink and ink artificially aged for 60 min. Ink slivers, 13 mm, were removed, placed in a vial, and extracted with 20 μL of toluene. Two 1-μL pipets were used to remove two aliquots of the extracted ink after extracting for 1, 3, 6, and 9 min. These eight aliquots were spotted on the TLC plate along a straight line. Densitometer readings of these provides a partial extraction curve. One minute later (at 10 min from the beginning) the remaining toluene was removed with a syringe and a minute later (at 11 min from the beginning) 20 μL of benzyl alcohol was added to the remaining residue. After 3 min of extraction in benzyl alcohol (at 15 min from the beginning) two 1-μL aliquots were removed and spotted along side the other eight spots. Stirring was done at 0.5, 2.5, 4.5, 6.5, 12, 13, and 14 min from the beginning.

*Experiment* 4—*Percent Extraction—Test Case (Natural Aging)*—Five entries known to have been written over a twenty-five-month period were obtained from the notebook containing naturally aged samples. The age of these samples were 10, 15, 20, and 25 months; an even spread. The TLC method was used to determine the percent extractions. In this case,

the extracting solvents were toluene and benzyl alcohol for the first and second extraction, respectively, and the corresponding extracting time was 10 min each. Their volume was 25 μL. The samples were 13-mm slivers which were placed inside a syringe. The extracting solvent was drawn from a vial into the syringe for extracting, flushed into the vial every two min, and emptied in the vial after extraction. Three 5-μL pipets were used to remove aliquots for TLC spotting and subsequent densitometric measurement.

Experiment 4 was designed and performed by Larry Olson, Document Examiner and Forensic Ink Chemist, IRS Document Laboratory, Chicago, Illinois.

## Results and Discussion

### *Use of Spectrophotometric Methods—Monitoring Fluorescence Extraction*

*Beer's Law*—The results of the measurements in Experiment 1 are tabulated in Table 1 along with their ratios. Each of the curves was formed by taking a fluorescence measurement every half minute of extraction over a period of 15.5 min. The comparison of the curves shows that after 4 min they are multiples of each other, that is, Beer's law holds.

TABLE 1—*Adherence to Beer's law. Fluorescence extraction of Fisher black ballpoint ink with 25% aqueous methanol.*

| Extraction Time, min | Relative Fluorescence Values | | | | |
|---|---|---|---|---|---|
| | 3 Discs | Ratio 3:6 | 6 Discs | Ratio 6:9 | 9 Discs |
| 1.0 | 9 | 2.4 | 22 | 0.95 | 21 |
| 1.5 | 12 | 2.1 | 25 | 1.1 | 28 |
| 2.0 | 14 | 2.0 | 28 | 1.3 | 36 |
| 2.5 | 17 | 1.7 | 29 | 1.4 | 42 |
| 3.0 | 18 | 1.7 | 31 | 1.5 | 46 |
| 3.5 | 19 | 1.7 | 32 | 1.6 | 50 |
| 4.0 | 19 | 1.8 | 34 | 1.6 | 53 |
| 4.5 | 19 | 1.8 | 35 | 1.6 | 56 |
| 5.0 | 20 | 1.8 | 36 | 1.6 | 57 |
| 5.5 | 20 | 1.9 | 38 | 1.6 | 60 |
| 6.0 | 21 | 1.8 | 38 | 1.6 | 61 |
| 6.5 | 21 | 1.9 | 39 | 1.6 | 62 |
| 7.0 | 21 | 1.9 | 40 | 1.6 | 64 |
| 7.5 | 22 | 1.8 | 40 | 1.6 | 65 |
| 8.0 | 23 | 1.8 | 41 | 1.6 | 65 |
| 8.5 | 23 | 1.9 | 43 | 1.5 | 66 |
| 9.0 | 23 | 1.9 | 43 | 1.6 | 67 |
| 9.5 | 24 | 1.8 | 44 | 1.5 | 68 |
| 10.0 | 24 | 1.8 | 44 | 1.6 | 69 |
| 10.5 | 24 | 1.9 | 45 | 1.5 | 69 |
| 11.0 | 24 | 1.9 | 45 | 1.6 | 70 |
| 11.5 | 25 | 1.8 | 45 | 1.6 | 71 |
| 12.0 | 25 | 1.8 | 46 | 1.5 | 71 |
| 12.5 | 27 | 1.7 | 46 | 1.5 | 71 |
| 13.0 | 26 | 1.8 | 46 | 1.6 | 72 |
| 13.5 | 26 | 1.8 | 47 | 1.6 | 73 |
| 14.0 | 26 | 1.8 | 48 | 1.5 | 73 |
| 14.5 | 26 | 1.8 | 47 | 1.6 | 74 |
| 15.0 | 27 | 1.8 | 48 | 1.6 | 75 |
| 15.5 | 27 | 1.8 | 48 | 1.6 | 75 |

Beer's law may very well hold also during the first 4 min of extraction; however, the discrepancy observed may be due to the extraction being faster than the measuring time. Hence, for better reliability, it is advisable to work with the values obtained after 4 min. Note that the ratios do reflect that six discs contain approximately twice as much ink as three discs, and nine discs contain approximately one and a half times the ink in six discs.

*Percent Extraction*—Table 2 summarizes the results of the fluorescence measurements from Experiment 2. This can be viewed as a three-dimensional graph of percent extraction as a function of solvent strength and age. The general trends observed are that each row of Table 2 represents a monotonically increasing solvent strength curve and each column represents a monotonically decreasing aging curve. The behavior of the aging curves with respect to solvent strength can be characterized by two parameters: their relative height and their curvature.

The relative height of an aging curve is the relative difference between the maximum value (at $T = 0$) and the minimum values (at $T = \infty$). The maximum difference (absolute and relative) appears to be at 25% methanol; above and below this it decreases. A measure of the curvature is the "half-aging" time, that is, the age at which one is halfway down on the aging curve. As expected, the "half-aging" time increases with solvent strength and thus supports the supposition that the leveling off age increases with solvent strength.

An important thing to mention here is that these results are for the Fisher black ballpoint ink which has a rhodamine-type fluorescent component. The fluorescence method used monitored only the extraction of this individual dye component. Consequently, what is true here, particularly the expected monotonic trends, may not be the case when multiple components are monitored (Eq 7). This will be treated later when a separation method is proposed.

### *Use of Spectrophotometric Methods—Monitoring Color Extraction*

Most inks do not contain fluorescent dyes like rhodamine, that is, dyes (1) which are highly fluorescent dyes and (2) whose fluorescence characteristic (green visible range) conveniently do not interfere with those of paper optical brightners (UV to blue visible range). The majority of writing ink dyes have either no fluorescence or low fluorescence in the UV or infrared (IR) region. As a result of this, monitoring of their color as they extract was investigated.

TABLE 2—*Percent fluorescence extraction versus solvent strength and ink age.*

| Time in Oven at 100°C | Percent Aqueous Methanol | | | | |
|---|---|---|---|---|---|
| | 12.5 | 25 | 50 | 75 | 100 |
| 0 | 6.56 | 30.1 | 90.2 | 97.7 | 100 |
| 5 | 3.71 | 16.4 | 89.5 | | |
| 10 | 3.33 | 15.6 | 88.7 | | |
| 15 | 3.18 | 13.1 | 88.0 | | |
| 20 | 3.87 | 12.8 | 83.5 | | |
| 25 | 2.96 | 10.3 | 84.8 | | |
| 35 | | 11.5 | 84.8 | | |
| 45 | | 10.6 | 82.7 | | |
| 55 | 2.56 | 9.17 | 83.5 | 98.5 | |
| 65 | 2.69 | 7.08 | 81.2 | | |
| 75 | | 6.48 | 87.2 | | |
| 80 | | 7.29 | 83.5 | | |
| 85 | | | 80.5 | | |
| 105 | | 7.52 | | | |
| 165 | | 6.99 | | | |

*Fluorescence Method for Inks Without Fluorescent Components*—For such inks a different approach is taken in using the fluorescence method. The extracting solution is *spiked* with a premeasured amount of a rhodamine-type dye. The extraction procedure is carried out as before but the measurement is that of fluorescence "robbing" by the extracting color. This "robbing" can be absorption of the excitation light (primary absorption) or of the emitted fluorescence light (secondary absorption) or both. Clearly, for this to work the extracting species must have absorption either at the excitation wavelength or the emission wavelength, or preferably, both. There is an increase in sensitivity when the extracting species absorbs both excitation and emission wavelength. In this method, one has to guard against possible oxygen fluorescence quenching (see below).

An equation can be simply derived (see Appendix A) which shows that the percent decrease from the initial fluorescence, that is, percent fluorescence "robbing," is directly proportional to the total absorbance (primary or secondary or both) of the extracting species,

$$\frac{F_{obs}(t) - F_{tot}}{F_{tot}} = 2.3[A_I(t) + A_{II}(t)] \tag{12}$$

where $F_{tot}$ is the initial, spiked, fluorescence, $F_{obs}(t)$ is the observed fluorescence after extracting for $t$ min, and $A_I(t)$ and $A_{II}(t)$ are the primary and secondary absorptions, respectively. Since we know that these absorbances are weak, the sensitivity of this spiking method is not as good as the direct fluorescence method. Thus, the concentration should be made greater by using more sample or decreasing the solvent volume. Note that this spiking method excludes studing inks containing any of the rhodamine-type dyes (such as the Fisher black ink).

To test the validity of Eq 12, a 25% aqueous methanol solution was spiked with rhodamine B and this was used to dissolve several inks (which must not contain Rhodamine B). The absorbance of each of the resulting colored solutions was measured using a UV-Vis spectrophotometer at 525 nm and at 552 nm to get $A_I$ and $A_{II}$, respectively. The fluorescence, $F_{obs}$, of the same colored solution was measured as well as that of the spiked solution, $F_{tot}$. Equation 12 was shown to be remarkably valid.

One of the problems encountered with the spiking method for obtaining extraction curves is that oxygen quenching is greater than the low fluorescence "robbing" caused by the low absorbance. Consequently, the reliability is poor. Besides reducing the oxygen quenching, greater sample size is needed to make the method tractable. For this reason, direct absorbance methods using microtechniques were also investigated.

*Direct Absorbance Methods*—With direct absorbance methods we are interested in monitoring the extraction of color. Consequently, it is not necessary to exclude inks containing any type of fluorescent dye. As can be surmised from Eq 12, a key difference between the spiking method and a direct absorbance method is that the former can measure essentially two absorbances and is thus potentially twice as sensitive as the latter.

Direct absorbance extraction measurements using UV-Vis spectrophotometry with microcells were investigated for obtaining extraction curves. The minimum volume of solvent required was about 40 μL, and thus sufficient sample (about 25 micro discs) was required to provide reliable absorbances. The extraction procedure was similar to that described above. The results were not satisfactory, though the general trends were observed. The difficulty could be due to problems associated with solution microspectrophotometry such as duplicating cell alignment and scattering.

Also, paralleling the previous method of successively spotting a TLC, successively injecting the extract into an HPLC (with and without a separating column) with a variable wavelength detector was tried for obtaining extraction curves. Again, general trends were obtained but the results were not satisfactory, perhaps because the procedure was not optimized.

*Change of Solvent*—When sequential solvent extractions were tried using the spiking fluorescence method, it was discovered that methanol, as the second extractor, was *not* an "all extracting" solvent for some dyes as it was for the rhodamine dye in the Fisher black ink. Also some very interesting observations involving the use of water-methanol mixtures as the first solvent led to the investigation of nonaqueous solvents as alternatives. These observations included the fact that methanol extracts more ink (Bic black in this case) from paper that has been wetted with at least a 25% methanol solution than one wetted with pure water. Also, the humidity in the room appears to affect the extraction efficiencies of nonrhodamine dyes when water is involved as a solvent.

Because of this, benzyl alcohol was tried as a second solvent. It quickly removes ink from paper and does not destroy the color. Since there are few solvents that are miscible with benzyl alcohol, the choice of the first solvent is limited. Toluene is a good choice not only because it is miscible but it makes the entire extracting system nonaqueous. These solvents, unfortunately, are not very suitable for direct or spiked fluorescence work.

Despite the difficulties encountered with the direct absorbance methods mentioned (UV-Vis and HPLC) to get extraction curves, they were used to perform sequential solvent extractions using both the water-methanol and toluene-benzyl alcohol systems. The results were not very encouraging because, besides the problems mentioned, the differences in the index of refraction of the solvents give unreliable absorbance values. This began the investigation of the TLC method discussed below as it does not depend on the extracting solvents, and thus, their indices of refraction. As will be seen, the TLC method gives very encouraging results.

From this it was decided that the direct fluorescence method using the water-ethanol system is valid from extracting single rhodamine-type dyes. In every other case, the TLC method using the nonaqueous toluene-benzyl alcohol system works well.

In concluding this part of the discussion, an interesting observation of the rhodamine- and nonrhodamine-type dyes in the Fisher black ink should be mentioned. A TLC plate is spotted with the methanol-water extracts of two inks of different age (after extracting for about 10 min). The plate is developed as usual to separate the dyes and the relative percent of the dyes is determined. Under UV examination, one can see that the extraction of the nonfluorescent colored dyes decreases more with age than that of the fluorescent rhodamine-type dyes. This will be treated later when a separation method is suggested to monitor individual dyes.

### *Use of the Chromatographic Method*

*Percent Extraction Curve*—Experiment 3 was conducted to show that a percent extraction curve contains information on both the rate and extent of extraction. The results appear in Table 3. For comparing rates of extraction, the $R$ ratio procedure is used (normalized column in Table 3*a*). Though there are only four points on the curve, the $L$th extraction time procedure, normally suited when more points on the extraction curve are available, is used to illustrate how it works (Table 3*b*) as an alternative way for comparing rates of extraction. Note, however, that the extent of extraction (Percent Extraction column in Table 3*b*) is *more sensitive* to aging than the rate of extraction measurements ($R$ ratios and $L$th extraction times).

*Natural Aging*—The results of Olson's measurements are listed in Table 4. As can be seen, the intrasample reproducibility is very good and the variances are nonoverlapping. Also, there is no indication that aging has leveled off at 25 months from the measuring time. Thus, for this ink on this notebook paper, the toluene/benzyl alcohol sequential solvent choice appears to be a good age discriminating system for an age bracket in excess of 25 months.

Olson also attempted analyzing these same inks using progressively stronger first solvents

TABLE 3a—*Percent extraction values.*

| Extraction Time (min) in Toluene | Densitometric Readings: Average 0 min | Average 60 min | Corrected 0 min | Corrected 60 min | Normalized[a] 0 min | Normalized[a] 60 min | Percent Extraction[b] 0 min | Percent Extraction[b] 60 min |
|---|---|---|---|---|---|---|---|---|
| 1 | 3.60 | 1.54 | 3.60 | 1.54 | 0.594 | 0.377 | 11.6 | 4.06 |
| 3 | 4.40 | 2.16 | 4.32 | 2.09 | 0.713 | 0.511 | 14.0 | 5.51 |
| 5 | 5.47 | 3.17 | 5.18 | 2.91 | 0.855 | 0.711 | 16.6 | 7.68 |
| 7 | 6.17 | 4.51 | 5.67 | 3.85 | 0.936 | 0.941 | 18.3 | 10.2 |
| 9 | 6.97 | 4.92 | 6.06 | 4.09 | 1.00 | 1.00 | 19.6 | 10.8 |

[a]These are the $R$ ratios; they are ordinates of the rate curve.

[b]The average densitometric readings of the benzyl alcohol extraction was 24.9 (for the 0-min ink) and 33.8 (for the 60-min ink). Thus, the densitometric reading of the total amount of ink sampled is 6.06 + 24.9 = 31.0 (for the 0-min ink) and 4.09 + 33.8 = 37.9 (for the 60-min ink).

TABLE 3b—*Approximate* L*th extraction times, in min.*[a]

| $0 < L < 1$ | Ink Age: 0 min in Oven | Ink Age: 60 min in Oven |
|---|---|---|
| $L = 1/2$ | 0.8 | 3.2 |
| $L = 3/4$ | 3.3 | 5.6 |
| $L = 9/10$ | 5.5 | 7.0 |

[a]Obtained by graphing the normalized values and extrapolating.

TABLE 4—*Percent extraction in natural aging.*

| Age in Months | Densitometric Readings: Solvent 1 Toluene | Solvent 2 Benzyl Alcohol | Sum | Percent Extraction |
|---|---|---|---|---|
| 25 | 2.8 | 20.8 | 23.6 | 11.9 |
| | 2.7 | 20.7 | 23.4 | 11.5 |
| | 2.7 | 20.1 | 22.8 | 11.8 |
| Average[a] | 2.7 | 20.5 | 23.2 | 11.7 |
| 20 | 4.0 | 22.4 | 26.4 | 15.2 |
| | 3.8 | 21.2 | 25.0 | 15.2 |
| | 3.7 | 17.7 | 21.4 | 17.3 |
| Average[a] | 3.8 | 20.4 | 24.3 | 15.9 |
| 15 | 4.5 | 19.1 | 23.6 | 19.1 |
| | 4.4 | 18.3 | 22.7 | 19.4 |
| | 3.9 | 16.0 | 19.9 | 19.6 |
| Average[a] | 4.3 | 17.8 | 22.1 | 19.4 |
| 10 | 5.5 | 16.0 | 21.5 | 25.6 |
| | 5.3 | 15.6 | 20.9 | 25.4 |
| | 5.1 | 14.8 | 19.9 | 25.6 |
| Average[a] | 5.3 | 15.5 | 20.8 | 25.5 |

[a]These averages are of the corresponding columns. The percent extraction computed using the average solvent 1 value and the average sum value are, in ascending order, 11.6, 15.6, 19.5, and 25.5.

in an effort to get a three dimensional graph or table like Table 2. The first solvents involved increased concentrations of benzyl alcohol in toluene. The results were not as expected, that is, the monotonic trends observed before did not necessarily occur. This indicated to us that, in the chromatographic method (which looks at the extraction of the unseparated dyes in the ink), using dilutions of a strong solvent as a first solvent creates problems—perhaps as a result of the unstable molecular complexes formed upon mixing—and pure solvents of varying strength, such as the aliphatic alcohol series, may be a better choice. We shall discuss this more when the choice of solvents is addressed.

*Direction of Extraction Efficiencies with Aging*

The expected trend of the three parameters considered with ink aging are:

1. For rates of extraction:
   (a) a decrease of the $R$ ratios; the older the ink is, the lower is the fraction extracted (of the total amount extracted) at a given time;
   (b) an increase in the $L$th extraction time; the older the ink is, the longer it takes to reach a given percent of extraction ($[L \times 100]\%$) of the total amount extracted.
2. For extent of extraction: a decrease in the percent of ink extracted (relative to the total amount of ink).

*Direction of Extraction Rates with Aging*—In every case listed on Table 3 the $L$th extraction time is higher for the older ink and the $R$ ratio is generally lower for the older ink. This is what one would intuitively expect as indicated above. This is also seen in Fig. 1; the fixed absorption ($L$th extraction time procedure) increases with age while the fixed extraction time ($R$ ratio procedure) decreases with age.

This is not always the case. In Fig. 1, the curve for $T_a$ may cross that for $T_b$ making one be above the other in one extracting region and the reverse in another extracting region. These discrepancies, when they occur, may be due to the way the individual dye components extract in the extracting solvent. In Appendix B we illustrate a case involving such crossing. Despite these discrepancies the method is still practical. The direction these measurements take with age can be determined by comparing ink samples of known age or by artificially aging the ink. However, as will be discussed later, the use of *separation methods* (for example, TLC or, if appropriate, HPLC) to monitor the individual dye extraction curves should be considered to resolve these directional discrepancies.

As an example of these discrepancies, the fluorescence method was used to obtain extraction curves (like Experiment 1) of inks of different artificial age (0, 20, 40, and 60 min in oven) using 0 and 25% aqueous methanol solutions. The extraction was carried out for 34 min. It was found that [*11*]:

1. The normalized rate curves (every ordinate of which is an $R$ ratio) *completely reversed* their order in going from extracting with pure water to 25% methanol solution! For the aqueous extraction, the order is: the 60-min curve is above the 0-min curve at all points; the 20- and 40-min curves are between these two (the 60- and the 0-min curves)—after 14 min of extraction, the 40-min curve is above the 20-min curve and before 14 min the reverse is the case, that is, there is a curve crossing at 14 min of extraction. For the 25% methanol extract, the order is reversed; it also includes the crossing at 14 min of extraction! Thus, the order is as expected when extracting with 25% methanol and one is looking past 14 min of extraction.
2. The $L = 0.9$ extraction time decreased with age when pure water was used as the extracting solvent and increased when methanol was mixed in. Thus, the order is as expected when extracting with 25% methanol and $L = 0.9$.

*Direction of Extent of Extraction with Aging*—All testing done with pure solvents (and solvent dilutions in the fluorescence of pure rhodamine) has shown that the percent of ink

extracted at a long extraction time decreases as the age of the ink increases (except at the leveling off region where the values fluctuate around the margin of error). A few tests have shown this to be true also at shorter extraction times. It thus appears that for two inks of different age, the entire percent of extraction curve of the older ink is below that of the younger one. When these curves are normalized by dividing by their ordinate at the final extracting time (in the first solvent), one obtains the mass invariant curves (or their modification) discussed before (Eqs 1 and 2). Again, these curves reflect only the rate of extraction, and as mentioned, the measurement of this rate does not necessarily decrease with age.

### *The Choice of Solvents*

The choice of solvents is one of the key elements and perhaps the most important one of this work. The direction to take in the choice of solvent has evolved from several experiments tried of which some are reported here. What is presented here is preliminary and shows where we currently stand. There is still more work to be done and it is hoped that this will stimulate further research in this area.

*Strength of Extracting Solvents*—To show that two inks are of different age, a solvent must be found that will distinguish their extraction efficiencies. A preliminary examination using the "Kikuchi"[4] spotting method [*12*] can point to the solvent of choice. Here a solvent is applied to an ink line using a small pipet and the dispersion of the ink is observed.[5] A solvent that shows a difference in the dispersion pattern of the two inks being compared is a candidate for performing the extraction measurements—particularly extent of extractions.

Preliminary studies [*9*] show that very weak extracting solvents can only differentiate inks written within days. As the solvent extracting strength increases, inks written within weeks, months and years, at most ten in some formulations, can be differentiated. The "strength" of these solvents is guided by their ability to discriminate age units (days, weeks, months, years); the "Kikuchi" spotting method mentioned above can be used to determine such strength.

This, of course, holds for the *unseparated* dyes in the inks. Individual dye components may extract quite differently—some faster than others in a given solvent (see below).

*Types of Extracting Solvents*—The choices of the extraction solvents include:

1. *Pure solvents* of varying extraction strengths such as water, aliphatic alcohols, pyridine, and benzyl alcohol. The latter two are perhaps the most efficient extractors of ball point inks.
2. *Dilution of strong solvents* using appropriate miscible solvents. Examples include aqueous dilutions of the aliphatic alcohols or pyridine and dilutions of benzyl alcohol with toluene.

*Aqueous Dilutions*—With the use of water as a pure solvent or diluent one has to consider the hygroscopic nature of paper. Thus, comparison of inks on the same document should be done during the same day, that is, while the document is under constant temperature and humidity conditions—or during equivalent conditions. This may also be necessary for nonaqueous solvents or their nonaqueous dilutions.

*Unseparated and Separated (Individual) Dye Components*—In the case of dilutions, unexpected trends in extraction efficiencies can occur as was indicated above in the natural aging experiment. We speculate that this is because the dye system considered is not an

[4]Dr. Kikuchi corresponded with one of the authors (AAC) in November 1974 supplying him with her excellent studies on ink aging of blue-black fluid inks and her thoughts on the aging of ballpoint pen inks. Her approach initiated the ideas presented in this paper.

[5]Kikuchi's principle observation, however, is the time it takes to achieve the dispersion; this time is a mass invariant measurement and is obviously related to our $L$th extraction time where $L$ is around 100% completion.

individual dye (as rhodamine was in Table 2). Also, mixing solvents may create weak hydrogen bonding complexes or associated molecular complexes. In an unseparated dye system, each of the individual dyes may have widely different extractabilities in each of the pure solvents and in their complex or complexes; this could cause the unexpected trends observed. Consequently, pure solvents of varying strength is recommended for monitoring unseparated dyes.

It was an afterthought from the results of this work that the extraction of individual dyes should be addressed. For this it is necessary to first separate the dyes.

*Modified TLC Chromatographic Method for Sequential Solvent Extraction*

The separation of dyes and studying their extraction can be incorporated easily in the sequential solvent extraction procedure that uses the TLC method. One simply develops the plate in solvent system I (ethyl acetate:ethanol:water = 70:35:30) and densitometrically analyzes each of the developed spots.

*Densitometry*—For each ink sampled there would be two sets of developed spots: one for the ink extracted in the first solvent and the other for the extraction of residual ink in the second (all extracting) solvent. The densitometric readings can be performed *along* (dye profile) and *across* the TLC development direction. For sake of example, suppose that there are three dyes extracted: Dyes A, B, and C. There would then be a total of six different dye spots. The amount of dye in each of these is related to the area under the densitometric curve.

Let us summarize at least three different ways of computing the percent of extraction of a given dye from these areas. Note that each of these percentages is mass invariant.

1. For Dyes A, B, and C, refer to the corresponding amounts extracted (the area under the densitometric curve) in the first extracting solvent as $a$, $b$, and $c$, respectively, and the amounts extracted in the second (completely extracting) solvent as $\bar{a}$, $\bar{b}$, and $\bar{c}$, respectively.

2. The percent of Dye A extracted in the first solvent *relative to* the total amount of ink extracted in this solvent is given by

$$\%\mathrm{A} = \frac{a}{a + b + c} \times 100$$

This mass invariant parameter is emerged entirely from separating the dyes extracted in the first solvent system. It resembles an extent of extraction type of parameter, but *there is no reference to the second solvent extraction.* It is a parameter worthy of investigation.

3. The percent of Dye A extracted in the first solvent (relative to the total amount of Dye A deposited) is given by

$$\%\mathrm{A} = \frac{a}{a + \bar{a}} \times 100$$

4. The percent of Dye A extracted in the first solvent (relative to the total amount of ink deposited) is given by

$$\%\mathrm{A} = \frac{a}{a + b + c + \bar{a} + \bar{b} + \bar{c}} \times 100$$

The latter two follow the traditional definition of percentage since they are percentages relative to the total amount of material deposited—total amount of Dye A in 3 and total amount of ink in 4.

5. Comparing these with what has been developed for the unseparated dye system we see that the percent of ink extracted in the first solvent (relative to the total amount of ink deposited) is given by

$$\%(A + B + C) = \frac{a + b + c}{a + b + c + \bar{a} + \bar{b} + \bar{c}} \times 100$$

which becomes, as expected,

$$\%(A + B + C) = \%A + \%B + \%C$$

as used in 4 above.

Note that using extracting solvents other than strong solvents is what permits observing differences in densitometric dye profiles of two inks which only differ in age. This will not happen when strong extracting solvents are used. This is why strong solvents are used for comparing static (compositional) characteristics of inks.

*Relation to the Kikuchi Method*—The modified method proposed serves as a generalization of the Kikuchi method mentioned above for choosing an extracting solvent. In the Kikuchi method one searches for a solvent that gives different dispersion patterns when the solvent is applied equally to lines of ink on paper which are of "equal intensity." The method described identifies analytically the dye which gives different extractions and thus can find discriminating solvents better without the need to assure equal sampling.

If no such solvent exists that can discriminate, then the two inks being compared are of the same age or they have leveled off in their aging and may be of the same or different age. To determine if leveling off has not occurred the inks have to be naturally or artificially aged to see if the aging is still occurring.

In summary, the advantages of separating the dyes from the extracted spots involve, as mentioned before, determining which is the most sensitive to aging in the extent of extraction. Also, this is expected to decrease and thus follow more the expected direction with aging than the individual dye rate of extraction as determined by the $R$ ratio or $L$th extraction time procedure.

## Conclusion

This preliminary work shows that the solvent extraction technique distinguishes inks of the same formula written at different times on the same paper. The approach of choice is the sequential extraction procedure which determines extent of extractions as percents of extraction. This seems to be more sensitive to aging than the procedures based on rate of extraction—the $R$ ratio and $L$th extraction time procedures.

At this stage, the TLC method is favored over the spectrophotometric methods. Finally, a TLC separation method is suggested for monitoring the percent extraction of individual dye components.

# APPENDIX A

### Derivation of the Fluorescence Absorbance Equation

Suppose a fluorescent compound in solution has maximum excitation at a given excitation wavelength and maximum emission at a given emission wavelength. Suppose also that this solution has an absorption spectra that shows absorption at the given excitation and emis-

sion wavelengths but is not from the fluorescent compound. Clearly, the first absorption reduces the excitation light and the second absorption reduces the fluorescence output.

The cuvette containing the fluorescent compound and the absorbing compound(s) can be viewed as three cuvettes: the first cuvette is between the excitation source and the second cuvette and contains just the compound(s) which absorb at the excitation wavelength, the second cuvette contains only the fluorescent compound, and the third cuvette is between the second cuvette and the fluorescence detector and contains only the compound(s) which absorb at the emission wavelength.

Let $P_0$ be the power of the excitation source. After it passes the first cuvette, it is reduced to $P_0[T_I]$ where $T_I$ is the transmittance of the solution in the first cuvette. This reduced power then enters the second cuvette producing a fluorescence given by

$$F' = F_{tot}[T_I] \tag{A1}$$

where $F_{tot}$ is the total fluorescence if the first cuvette did not exist. This fluorescence light then enters the third cuvette which reduces it to the final observed fluorescence,

$$F_{obs} = F'[T_{II}] \tag{A2}$$

where $T_{II}$ is the transmission of the solution in the third cuvette. Note that $F'$ would have the fluorescence if the third cuvette did not exist. Thus the final observed fluorescence is

$$F_{obs} = F_{tot}[T_I \times T_{II}] \tag{A3}$$

Next we borrow some ideas used in the classical derivation of the equation relating fluorescence intensity with the concentration of the fluorescent compound [*13*]. By Beer's law, transmittance is 10 to the negative power of the absorbance. Thus, $T_I \times T_{II}$ becomes 10 to the negative power of $[A_I + A_{II}]$. For absorbances less than 0.05 absorbance units, the Taylor series expansion yields,

$$T_I \times T_{II} = [1 - 2.3(A_I + A_{II})] \tag{A4}$$

Thus,

$$[F_{obs} - F_{tot}]/F_{tot} = 2.3[A_I + A_{II}] \tag{A5}$$

which is the desired result, Eq 12 in the text.

# APPENDIX B

### Crossing of Mass Invariant Curves—An Example

Consider an ink having only two dye components. The extraction curve for this ink is the sum of the individual extraction curves for each of the two dyes,

$$E(T; t) = E_1(T; t) + E_2(T; t) \tag{B1a}$$

$$= E_1(T; \infty)X_1(T; t) + E_2(T'; \infty)X_2(T'; t) \tag{B1b}$$

The mass invariant curve for this ink is the weighted sum of the individual mass invariant curves for each of the two dyes,

$$X(T; t) = fX_1(T; t) + (1 - f)X_2(T; t) \tag{B2}$$

where, $0 < f < 1$ is the fraction,

$$f = E_1(T; \infty)/E(T; \infty) = E_1(T; \infty)/[E_1(T; \infty) + E_2(T; \infty)] \tag{B3}$$

Clearly, $(1 - f)$ is the corresponding fraction for the second dye.

Assume that the second dye extracts very fast, for example, over 90% is extracted after the first minute of extraction. Furthermore, assume this is independent of age, that is, age does not progressively slow the extraction of this dye. This means then that for $t > 1$ min,

$$\text{ASSUMPTION I: } X_2(T; t) \cong X_2(T'; t) \cong 1 \tag{B4}$$

where $T$ and $T'$ are two different ages. Here we can arbitrarily take $T$ to be the younger age and $T'$ to be the older age. We thus have,

$$\text{New: } X(T; t) = fX_1(T; t) + (1 - f) \tag{B5}$$

$$\text{Old: } X(T'; t) = f'X_1(T'; t) + (1 - f') \tag{B6}$$

For these to cross, a $t$ must exist such that,

$$X(T; t) = X(T'; t) \tag{B7}$$

or,

$$fX_1(T; t) + (1 - f) = f'X_1(T'; t) + (1 - f') \tag{B8}$$

This becomes,

$$fe_1(T; t) = f'e_1(T'; t) \tag{B9}$$

where we have used the definition,

$$e(T; t) = 1 - X(T; t) \tag{B10}$$

Thus, for crossing to occur,

$$(f/f') = [e_1(T'; t)/e_1(T; t)] \tag{B11}$$

Assume that the individual mass invariant curve for the first dye decreases with age which is the expected direction,

$$\text{ASSUMPTION II: } X_1(T'; t) < X_1(T; t) \tag{B12}$$

Then,

$$e_1(T; t) < e_1(T'; t) \tag{B14}$$

Thus, for crossing to occur it is necessary that,

$$f' < f \tag{B15}$$

which means that the fraction $f$ (of the first dye) decreases with age. Note that this is at the expense of the fraction $(1 - f)$ (of the second dye) increasing with age.

We must now show that a extracting time $t$ exists where the curves $X(T; t)$ and $X(T'; t)$ cross.

Since a mass invariant curve $X(T; t)$ is a monotonically increasing function of $t$ with an asymptote of 1, the function $e(T; t)$ begins at 1 and monotonically decreases to its asymptote of 0. Because of this and Eq B14, the right hand side of Eq B11 begins at 1 and increases without bound with $t$. Because of Eq B15, the lefthand side of Eq B11 is greater than 1. Thus, there exist a $t$ so Eq B11 is satisfied.

We should note that in the exponential model mentioned in the text, the function $e(T; t)$ defined in Eq B10 is,

$$e(T; t) = \exp[-\beta(T)t] \tag{B16}$$

where $\beta(T)$ is the extraction rate constant. Compare this with Eq 8b in the text. It is related to the 1/2 extraction time, $t_{1/2}(T)$, by

$$\beta(T) = \ln 2/t_{1/2}(T) \tag{B17}$$

We did not need to use the exponential model to prove that crossing can exist. However, it provides a working model for testing conjectures. Using this model, the following can be shown for an ink with two dyes:

Suppose

$$E(T; t) = 4X_1(T; t) + 3X_2(T; t)$$

and

$$E(T'; t) = 1X_1(T'; t) + 2X_2(T'; t)$$

Note that this has the expected decrease with age of the individual extent of extraction for the two dyes.

Then the corresponding mass invariant curves become

$$X(T; t) = [4/7]X_1(T; t) + [3/7]X_2(T; t)$$

and

$$X(T'; t) = [1/3]X_1(T'; t) + [2/3]X_2(T'; t)$$

Suppose that for the second dye, half of total that can be extracted (value of 3 for the fresh ink and value of 2 for the older ink) is extracted in 15 s and this is the same for the fresh and old ink. That is, for this dye $t_{1/2}(T) = t_{1/2}(T') = 1/4$ min. Thus, by the end of 1 min of extraction 93.75% of the dye is extracted and essentially Assumption I is satisfied, Eq B4.

Suppose $t_{1/2}(T) = 5$ min and $t_{1/2}(T') = 8.18$ min for the first dye. That is, the aging caused the half extraction time to increase by 64%. An increase is the expected direction and satisfies Assumption II, Eq B12.

Then the two mass invariant curves cross at 10 min. After this time, the curve for the new ink is above that of the old and before that time, the reverse is true.

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