Arie Zeichner,[1] Ph.D.; Nadav Levin,[1] M.Sc.; Asne Klein,[1] M.Sc.; and Yehuda Novoselsky,[1] B.Sc.

Transmission and Reflectance Microspectrophotometry of Inks

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ABSTRACT: Various inks were shown to obey the Beer-Lambert Law as deposits on glass slides. However, spectra of ink traces on paper are subject to scattering and bronzing interferences which cause deviations from the law. Theoretical considerations and experimental results show that there is an inherent advantage of the transmission method over the reflectance method since the former does not suffer from bronzing interference. The deviations from the law may be considerably lower using transmission microspectrophotometry of single inked fibers in a mounting medium instead of the nondestructive transmission method because of large variations in the opacity of paper substrates. In any case, when measuring spectra of ink traces on paper, care must be taken when applying the Beer-Lambert Law to normalize spectra to eliminate concentration differences and substrate background contributions.

KEYWORDS: questioned documents, inks, spectroscopic analysis, microspectrophotometry

In recent years, microspectrophotometry has become an important technique for the comparison of small quantities of colored materials, for example, paints, fibers, inks, and so forth [*1-6*].

It was observed that the bronzing effect of inks on paper substrates (appearance of a reddish metallic sheen in the ink trace) may interfere substantially in the reproducibility of their reflectance spectra [*2-4*]. To overcome this problem, it was proposed either to select measurement areas that have adequate appearance of color with no "bronze" regions, or to place small samples of ink stained fibers in a suitable mounting medium and to record the transmission spectra [*2-4*].

It seems, however, that not enough attention was paid to scattering as a potential interference in obtaining reproducible spectra of inks on paper, in the reflectance as well as in the transmission modes.[2]

In this work we have studied the influence of scattering and bronzing in microspectrophotometry of inks on paper in view of the existing theories regarding light transfer in nonhomogenous media [*7-9*]. In particular, the influence of these effects on the quantitative comparison of spectra based on the validity of the Beer-Lambert Law (for example, computer software supplied with the Docuspec TM/1 microspectrophotometer) was examined.

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[1]Head and scientific officers, respectively, Toolmarks and Materials Laboratory, Division of Criminal Identification, Israel National Police, Jerusalem, Israel.

[2]If not stated otherwise, the meaning of reflectance throughout the paper will be diffuse reflectance.

Theory

The light passing through a nonhomogenous medium may be attenuated by a combination of processes including scattering, specular reflection, diffraction, dispersion, refraction, and absorption [*10,11*].

The Kubelka-Munk theory is the most widely used model that describes quantitatively the behavior of light in diffusing media [*7,8*]. This phenomenological model involves a simultaneous solution of two differential equations of the first order, which describe the diminution of light intensity, within the sample, as a result of scattering and absorption:

$$dI = -(k + s)Idx + sJdx \tag{1}$$

$$dJ = (k + s)Jdx - sIdx \tag{2}$$

where I is the light intensity in depth x of the sample (I is in the same direction as the incident intensity I_0 at $x = 0$), J is the backscattered intensity (opposite direction to I) in the same depth x, k is the absorption coefficient, and s is the scattering coefficient.

The term scattering in the model, encompasses, in fact, all the processes mentioned above except absorption. Through the following discussion we shall also use this term in the same sense.

Although the validity of the model is subject to various assumptions such as diluted systems, weak absorbing substances, uniform particle size, independence of the scattering coefficient on wavelength, and so forth, conditions that are far from being satisfied in the case of ink traces on paper, nevertheless, it may serve to assess the general effect of scattering on the spectra of inks on paper. Note that in the limit of no scattering, the solution of the differential equations is the Beer-Lambert Law:

$$A = \log I_0/I = kt = \epsilon ct \tag{3}$$

where A is absorbance, ϵ is the molar extinction coefficient, c is the concentration of the absorbing material, and t is the thickness of the sample.

In a case where scattering is not negligible and the layer is not infinitely thick, the expressions for transmission (T) and reflectance (R) are quite complicated and may be found elsewhere [*7,8*].

The Kubelka-Munk Model theory does not take into account the contribution of specular reflection to the measured reflectance. However, in cases where the particle size of the sample is relatively large, or especially in the case of strong absorbers, the contribution of specular reflectance may be very large [*7-14*]. The specular reflectance is governed by the Fresnel equations, which in the simple case of perpendicular incidence become [*7,8*]:

$$R = \frac{(n_1 - n_0)^2 + (n_1\kappa_1)^2}{(n_1 + n_0)^2 + (n_1\kappa_1)^2} \tag{4}$$

where n_1 is the refractive index of the absorbing medium and n_0 is the refractive index of the nonabsorbing medium (usually this medium is air and then n_0 is approximately 1), κ is the absorption index and is related to the absorption coefficient (k) in the following way:

$$k = \frac{4\pi n\kappa}{\lambda_0} \tag{5}$$

(λ_0 denotes the wavelength in vacuum).

In the absence of absorption, the specular reflectance is reduced, in air, to:

$$R = \frac{(n - 1)^2}{(n + 1)^2} \tag{6}$$

and, therefore, nonabsorbing materials generally reflect only a small fraction of the incident radiation. For strongly absorbing materials that satisfy the condition $n_1\kappa_1 \gg n_1$ [7], the equation may approach unity, for instance, in the case of metals. This is also generally the case for radiation that is not perpendicularly incident. Usually, one defines a strong absorber [8] as a sample for which $\bar{t} < \lambda$; and similarly for a weak absorber, $\bar{t} > \lambda$, where $\bar{t} = 1/\kappa$; $\bar{t}$ is defined as the mean depth of penetration of light into the layer of the sample. This means that for a strong absorber a large part of the light is absorbed within a distance of about a wavelength from the upper surface of the absorbing layer.

The maximum specular reflectance as a function of the wavelength occurs near the absorption maximum. This maximum does not generally coincide with the absorption maximum, however, since the index of refraction changes rapidly ("anomalous dispersion") in the vicinity of the absorption band. This change is not symmetric about the absorption maximum, the refractive index peaks being on the high wavelength side [7–12]. The maximum of specular reflectance in insulators is shifted slightly towards a higher frequency relative to the absorption maximum [8,10]. Therefore, the color of light reflected specularly from the surface of strongly absorbing materials is approximately complementary to the color of the light transmitted by it. This is the origin of bronzing that occurs in the case of many ink traces on paper, especially dark blue and black ones [2,3], as well as paints [13,14].

Experimental Procedure

Instrumentation

The system used was the Docuspec TM/1 computerized microspectrophotometer (Nanometrics, Inc.) which includes an Olympus BHT microscope with quartz-halogen lamps: 50 W for reflectance and 20 W for transmission work. The instrument is equipped with a variable measuring aperture and its wavelength range is from 380 to 764 nm. The software of the system is described elsewhere [15,16]. Here we only wish to mention that the software normalizes the unknown spectrum before its comparison to standards in the memory. The normalization is carried out to eliminate effects resulting from concentration and thickness differences and is obviously based on the validity of the Beer-Lambert Law. The degree of similarity in color of the unknown and the standard is reported as a match number (m.n.) that ranges from 0.00 (no difference) to 100.00 (maximum difference). The m.n. threshold value may be selected as a tolerance to accommodate differences which are acceptable for concluding that there is a match. According to the instruction manual the power-up value of 0.25 is often selected. However, the threshold value that is acceptable will vary with sample types. For instance, if the sample is uniform in color and texture like plastics, then a low match threshold such as 0.1 can be used.

Sample Preparation and Measurement of Spectra Using the Docuspec TM/1 Software

Twenty-seven inks, mostly from blue ballpoint pens, were examined to see whether their transmission spectra on glass slides obey the Beer-Lambert Law. The ballpoint pen inks and rolling ball pen inks were deposited on glass slides by writing many strokes with the pen on a polyethylene plastic sheet and pressing it on the slide. The permanent, fiber tip pen inks were deposited by writing directly on a glass slide. In both cases, a variable surface concentration of ink on the slide was obtained.

A ×20 objective lens was used to record spectra and the measurement area was usually approximately 20 by 40 μm. For each ink, an area was selected to give maximum absorbance in the range of 0.5 to 1.5 optical density (O.D.) and the obtained spectrum was stored as a standard in the memory. Subsequent spectra were recorded from five varying areas of the same ink deposit to cover a large absorbance range and were compared to the standard using the Docuspec software.

Areas of the ink deposits on glass slides were scratched to produce scattering, and spectra in the scratched areas were also recorded and compared to the transmission spectra obtained from the ink traces on paper.[3]

Permount® (Fisher Scientific Company) was used as a mounting medium to study its influence on the scratched areas of ink deposits on slides and in the ink traces on paper. The refractive index of Permount is 1.567 [*17*] and of cellulose about 1.53 [*18*].

For the study of the bronzing effect, the scratched and unscratched areas of ink deposits on glass coverslips and ink traces on paper were examined, and their spectra were recorded in the dark field (diffuse reflectance) and bright field (specular reflectance) modes. White paper was inserted under the coverslips to simulate better the situation of ink traces on paper.

Since it turned out that all of the examined inks (in the form of deposits on slides) practically obeyed the Beer-Lambert Law (see next section), and we had at our disposal a considerable number of blue ballpoint pen inks, they were chosen for a more comprehensive study as follows: four pairs of blue inks (the inks in each pair being very similar but distinguishable in their transmission spectra obtained from deposits on glass) were selected to examine the reproducibility of their spectra as traces on paper and whether the inks in each pair could still be discriminated when they were in the form of traces on paper. Two methods were used: (1) direct nondestructive transmission microspectrophotometry and (2) transmission microspectrophotometry of small samples of ink stained paper fibers in mounting medium as described elsewhere [*2,3,5*].

For each method and ink, a standard was stored in the memory according to the guidelines, as described previously, for ink deposits on slides. Also, areas having high local absorbance were omitted to refrain from too large a contribution of scattering. Owing to a poorer reproducibility in the case of ink traces on paper than ink deposits on slides, to improve statistics, spectra were recorded from ten different areas (range of maximum absorbance 0.5 to 1.5 O.D.) and were compared to the standard of the same ink and the standard of the other ink in the pair.

The possible influence of the type of paper on the obtained spectra was examined in the case of two different blue ballpoint pen inks by comparing their spectra on the white paper to those on a brown cover paper using Method 2. A brown cover paper was chosen for the comparison because of its relatively high absorbance.

Results and Discussion

All the inks examined in this study practically obeyed the Beer-Lambert Law when they were deposits on glass slides. The average m.n. was 0.03 ± 0.01 and typical maximum m.n. did not exceed 0.1.

During storage, the ink deposits on glass began to exhibit areas of scattering and bronzing, probably as a result of continuous drying and formation of areas of particulate nature.

Figures 1 and 2 show examples of transmission spectra of blue and black ballpoint pen inks for different absorbance ranges. Note that Spectrum c in Fig. 1, although recorded in the diffuse reflectance mode, is practically a transmission spectrum since the ink deposit obeyed the Beer-Lambert Law and a white paper under the coverslip served as a reflecting surface.

[3]If not stated otherwise, a groundwood-free white paper was used in the study.

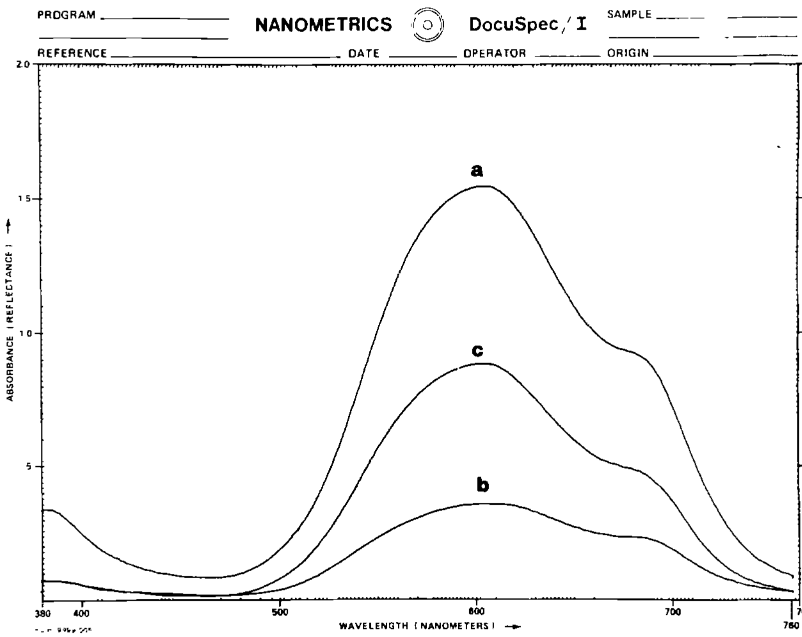

FIG. 1—*Spectra of a blue "Pelican" ballpoint pen ink as a deposit on a glass slide:* a *and* b *are transmission spectra and* c *is a diffuse reflectance spectrum of the ink deposit when a white paper is inserted under the slide. The three spectra match each other with m.n. less than 0.07.*

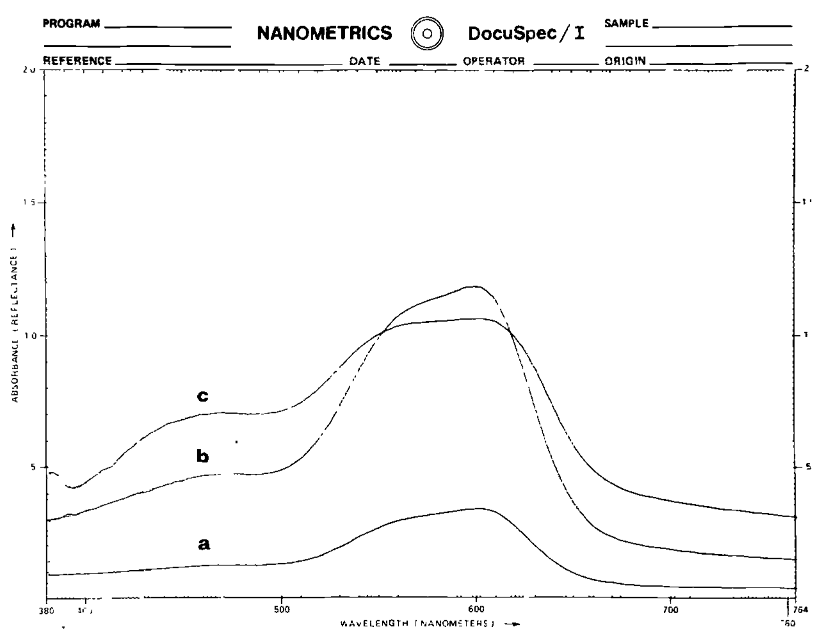

FIG. 2—*Transmission spectra of a black "Stick 433F" ballpoint pen ink:* a *and* b *are spectra of the ink as a deposit on a glass slide and* c *is a spectrum of the ink trace on a white paper.*

Scratching the ink deposits resulted in scattering, causing broadening of the transmission spectra as is generally predicted by the theory [7,8]. Such spectra could be matched to the transmission spectra of the same ink on paper (Figs. 2 and 3). At the same time, the reproducibility, based on the validity of the Beer-Lambert law, became poorer.

Many of the inks exhibited significant bronzing effects. Figures 4 to 6 show spectra of some inks demonstrating this effect. It may be seen that, as in the case of transmission spectra, the reflectance spectra of the ink traces on paper may be matched to those obtained from the scratched areas of the ink deposits on the glass slides. On the other hand, they show similarity to the specular reflectance spectra of the ink deposits on glass slides, the difference being due to scattering in the diffuse reflectance spectra and possible dependence of the specular reflectance on the angle of incidence. It is worthwhile to note that the black ink (Figs. 2 and 5) has a blue tint in transmission when it is a deposit on a glass slide. However, in scratched areas of the deposit and as a trace on paper it has a black appearance as a result of broadening of the absorbance in the visible spectrum, especially in the reflectance mode (Fig. 5).

It should be pointed out that bronzing does not occur in the ink deposits on glass when they are examined in the dark field reflectance mode, since there are not areas of the deposit at such angles that the light reflected from them specularly may be collected by the objective of the microspectrophotometer. However, scratching of the deposit causes many of its areas to be aligned at such angles that the light reflected from them specularly may be collected by the objective. These areas exhibit bronzing. Nonglazed papers are rough enough so that their microscopic areas are aligned at such angles as to exhibit bronzing. On the other hand, glazed papers are sufficiently smooth to prevent bronzing in diffuse reflectance.

Figure 7 shows reflectance spectra of blue-green ink that is not strongly absorbing and therefore does not exhibit bronzing. It may be seen that in this case there is a much smaller

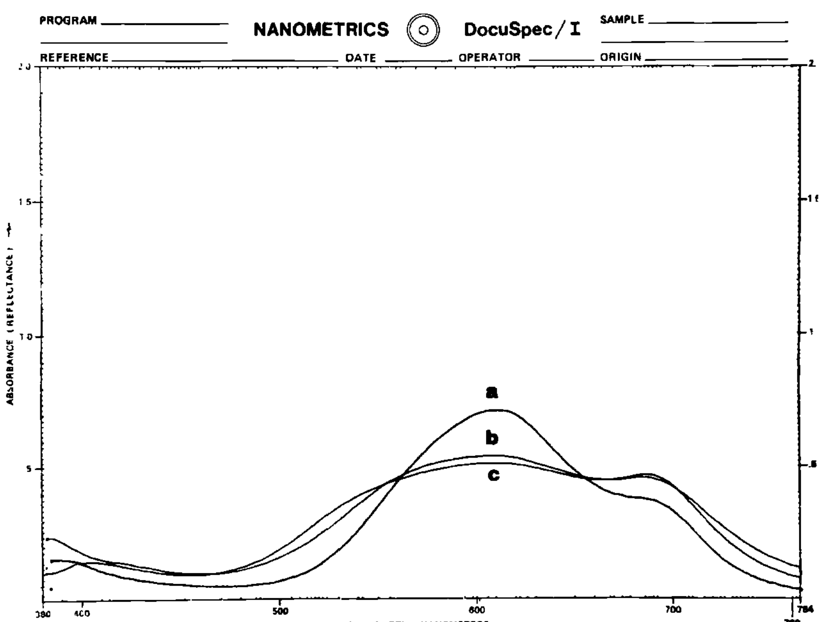

FIG. 3—*Transmission spectra of the same ink as in Fig. 1.* a *is a spectrum of the ink deposit on a glass slide,* b *is a spectrum of the ink on white paper, and* c *is a spectrum of the ink in a scratched area of the ink deposit on a glass slide.*

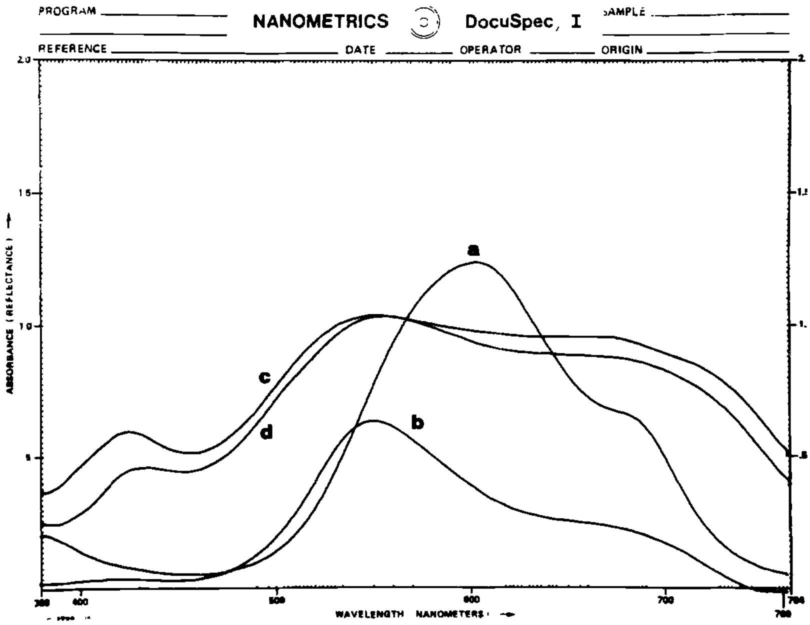

FIG. 4—*Spectra of the same ink as in Fig. 1:* a *is a transmission spectrum of the ink deposit on a glass slide,* b *is a specular reflectance spectrum of the deposit,* c *is a diffuse reflectance spectrum of a scratched area in the deposit, and* d *is a diffuse reflectance spectrum in the bronze areas of the ink trace on paper.*

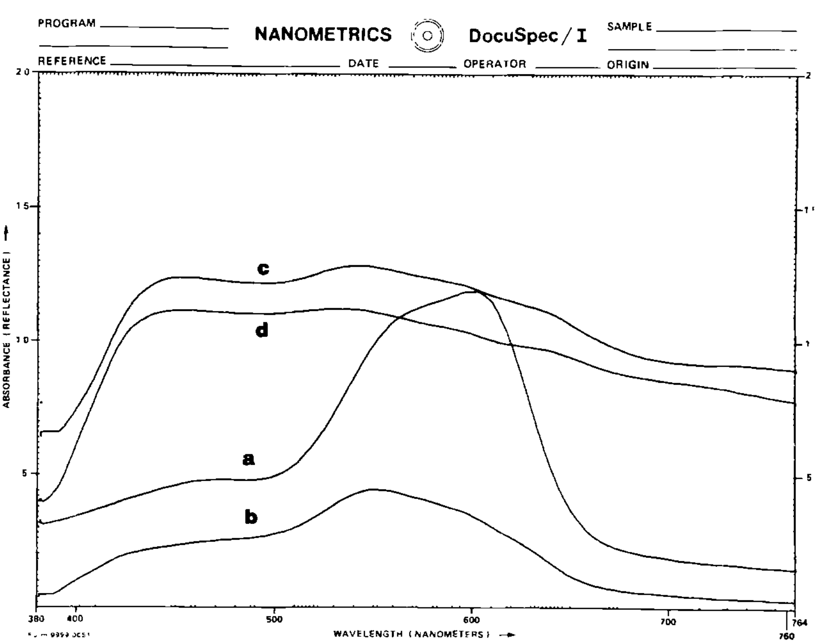

FIG. 5—*Spectra of the same ink as in Fig. 2, in the same modes as in Fig. 4.*

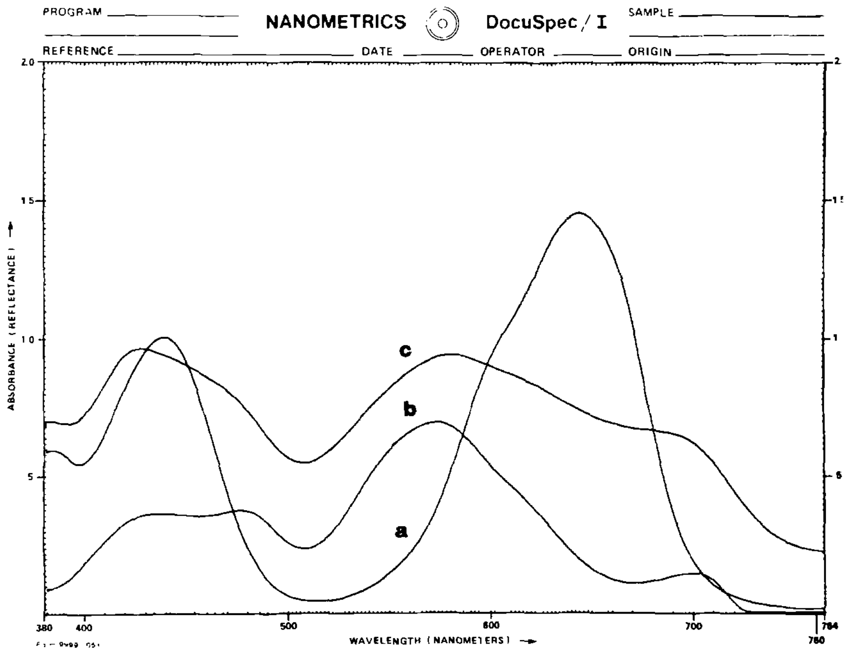

FIG. 6—*Spectra of a green "Faber Casell" fiber tip permanent ink* a *and* b *are in the same modes as in Fig. 4, respectively, and* c *is the same mode as* d *in Fig. 4.*

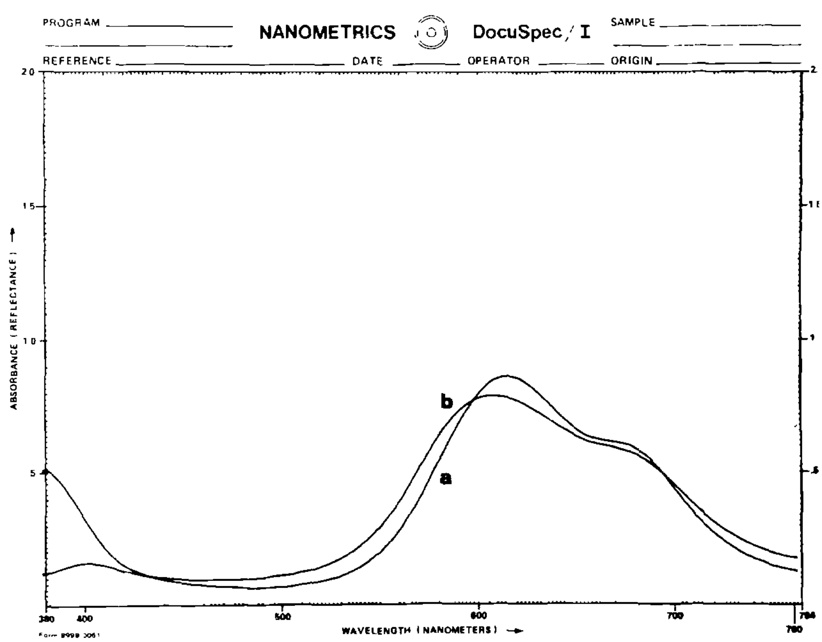

FIG. 7—*Spectra of a blue-green "Stick 433F" ballpoint pen ink.* a *is a transmission spectrum of the ink deposit on a glass slide and* b *is a specular reflectance spectrum of the deposit normalized to spectrum* a.

difference between the bright field reflectance spectrum and the transmission spectrum of the ink deposit than in the cases of inks exhibiting bronzing (Figs. 4 to 6).

Using a mounting medium did not solve significantly the problem of scattering in the case of strongly absorbing inks. For example, Fig. 8 shows that the addition of a mounting medium on the ink trace hardly changed the spectrum that was obtained without it. Similarly, adding Permount to the scratched areas of ink deposits on slides does not affect significantly the spectra. Even using single inked fibers in a mounting medium does not change the results. Figures 9 and 10 show examples of the spectra of single inked fibers in comparison with the transmission spectra of ink deposits on glass, in the case of highly absorbing inks. These results may be explained by considerably high internal specular reflections (Eq 4) and high dispersion ("anomalous dispersion") for strongly absorbing materials, both of which may contribute significantly to scattering. Support for this assumption is provided by spectra of single inked fibers in Permount in the case of low absorbing blue-green ink (Fig. 11). It may be seen that in such cases a mounting medium may reduce scattering significantly.

The spectra reproducibility (based on the Beer-Lambert Law) of the single inked fibers was found to be considerably worse than in the case of ink deposits on slides. However, in a situation where paper fibers are not tinted, the reproducibility may still be quite satisfactory; on average, a m.n. of about 0.1 ± 0.05 may be attained. Note this observation, since the above variation is much less than the difference (m.n. approximately 1) between the inked fibers' spectra and the spectra of the ink deposits on glass as was shown in Figs. 9 and 10. The following explanation may account for these results. Referring back to the Kubelka-Munk theory (Eqs 1 and 2), it may be assumed that in the case of single inked fibers the scattering medium is sufficiently thin so that $J \ll I$, although "s" is not negligible. Neglecting J in Eq 1, we obtain:

$$dI = -(k + s)Idx \qquad (7)$$

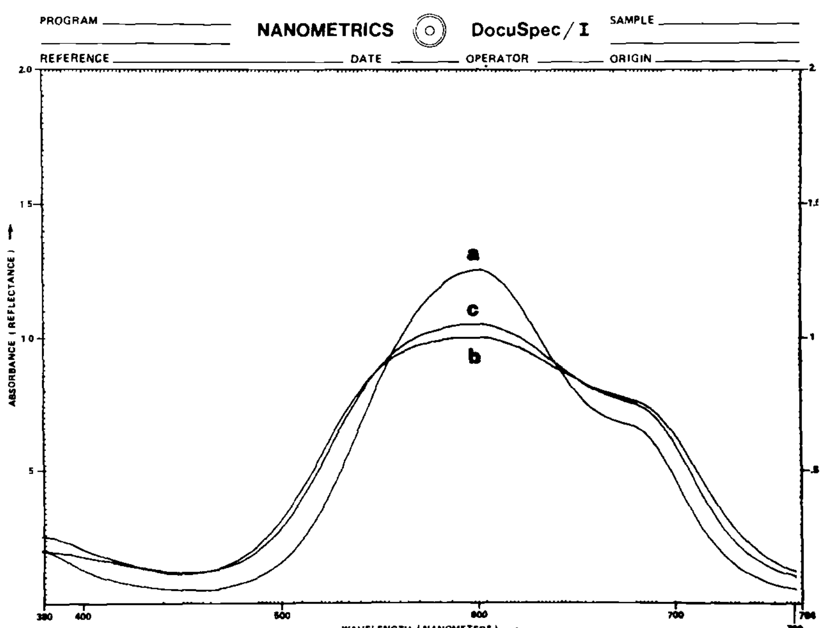

FIG. 8—*Transmission spectra of the same ink as in Fig. 1:* a *is a spectrum of the deposit on a glass slide.* b *is a spectrum of the ink on a white paper.* c *is the spectrum of the same area on paper (as in* b*), following the addition of Permount.*

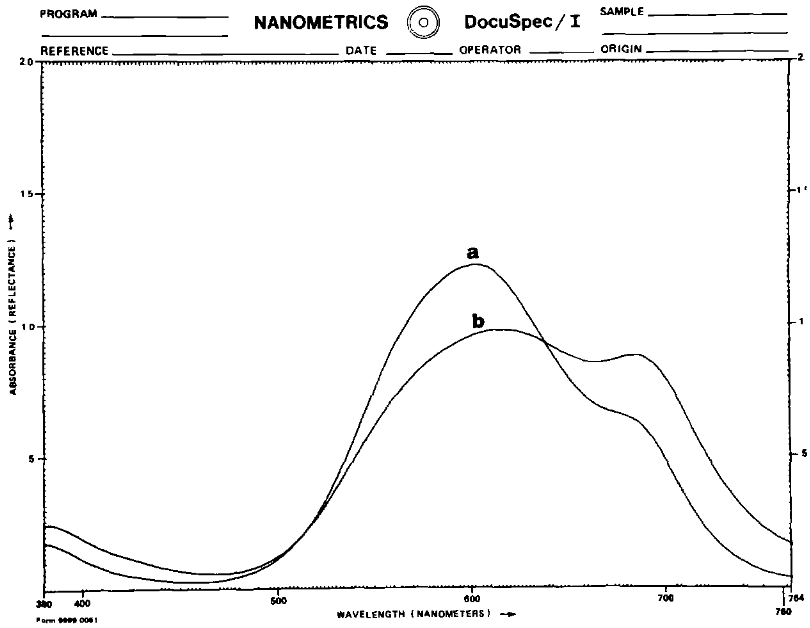

FIG. 9—*Transmission spectra of the same ink as in Fig. 1:* a *is a transmission spectra of the deposit on a glass slide,* b *is a spectrum of a single inked fiber in Permount normalized to spectrum* a.

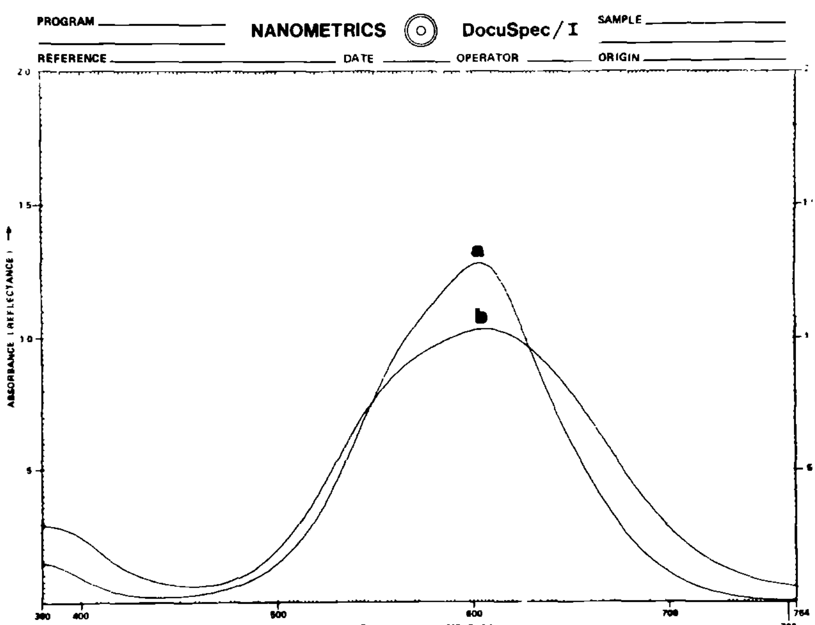

FIG. 10—*Transmission spectra of a blue "Stick 433F" ballpoint pen ink in the same modes as in Fig. 9.*

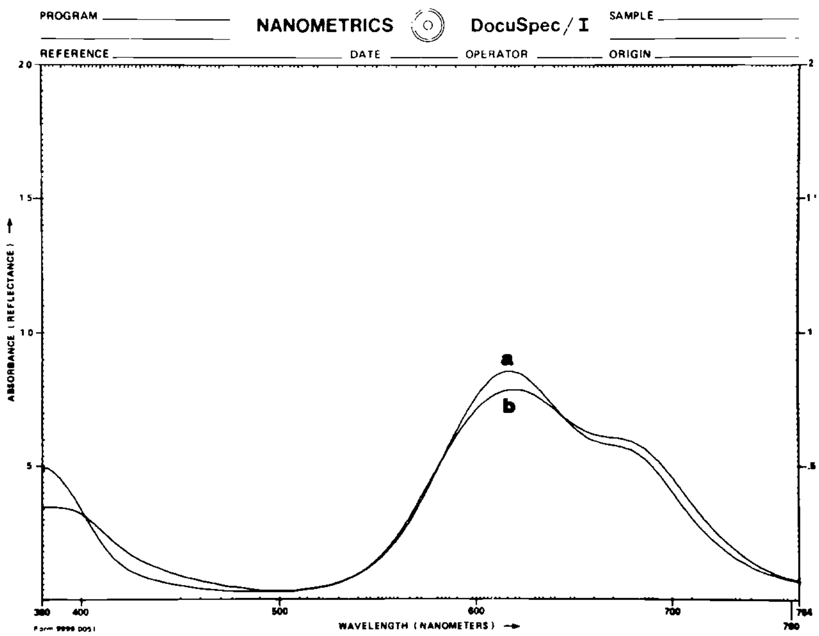

FIG. 11—*Transmission spectra of the same ink as in Fig. 7 in the same modes as in Fig. 9. respectively. The m.n. of spectrum* a *to* b *is 0.07.*

or

$$A = \log I_0/I = (k + s)t \tag{8}$$

which is the Beer-Lambert Law with the effective absorption coefficient of "$k + s$" (instead of "k" for ink deposits on glass) which causes the flattening of the spectra.

Using nondestructive transmission microspectrophotometry reduces significantly the reproducibility of the spectra. Even on the groundwood-free white paper the variation in opacity may be very large; the transmission of the least transparent area may be less than half compared to that of the most transparent one. It was found that the variation in reflectance of such paper is significantly less then in transmission. The average range of m.n. obtained on such paper for the same ink in transmission is about 0.3 ± 0.2.

The very similar inks in each pair of the selected four pairs were easily distinguished as deposits on glass slides by distinct features of the spectra such as the number of maxima, the wavelengths of maxima and minima, and so forth, and also by m.n. as is shown in Figs. 12 and 13 for two of the pairs. It was considerably more difficult to differentiate between them by their spectra using the method of single inked fibers in Permount. Actually, the differences could be detected, primarily by distinct features, rather than by variation in m.n. as a result of the reduced reproducibility of the spectra. Thus, in the case of the ink pair shown in Fig. 12, it was not possible to distinguish between the inked fibers by means of the m.n. However, distinction could be detected by the consistent difference in the wavelength of the minima appearing at about 650 nm, which was also observed in the spectra of the ink deposits. In the case of inks whose spectra are shown in Fig. 13, the discrimination could be detected by the shoulder at about 690 nm appearing consistently in the spectra of one of the inks. The spectral difference between the two inks in this case, as judged by the m.n., could be assessed with substantial difficulty.

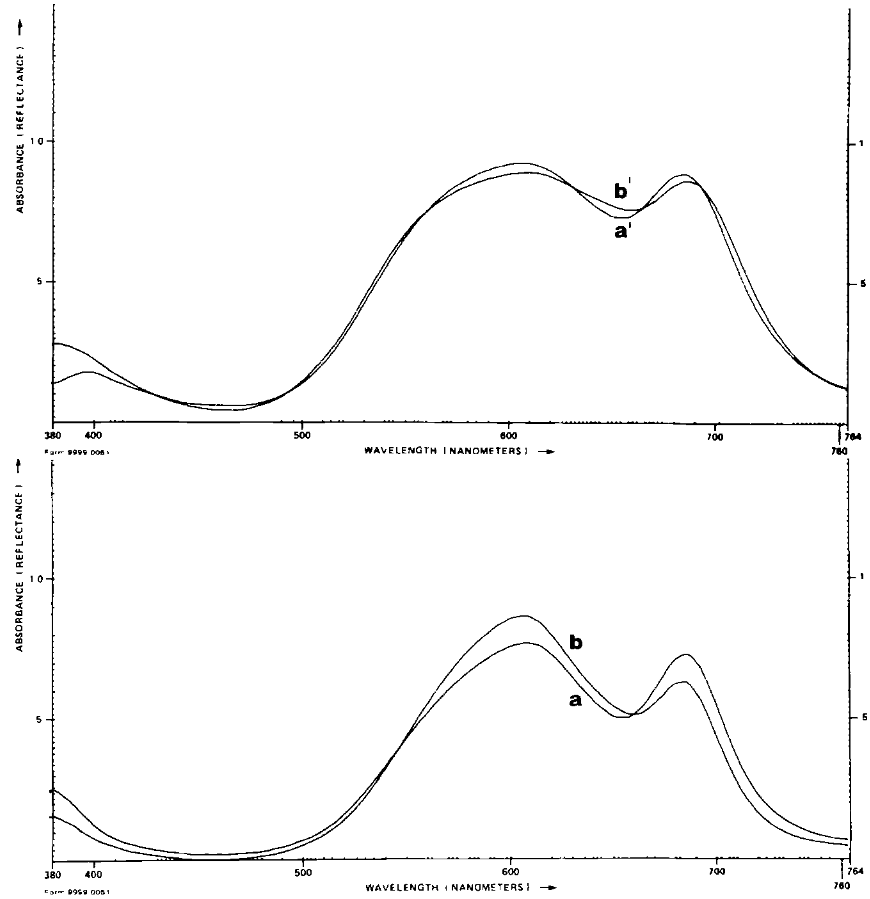

FIG. 12—*Transmission spectra of two very similar blue ballpoint pen inks: "Parker Medium"* (a,a') *and "Lamy Exact M16"* (b,b'). a *and* b *are spectra of the ink deposits on glass slides and* a' *and* b' *are spectra of single inked fibers in Permount.*

Using a brown cover paper instead of white paper did not change substantially the shape of the ink spectra; the only difference was the reduction in the reproducibility of the obtained spectra. In the case of the two examined blue ballpoint pen inks referred to in Figs. 9 and 10, using the method of single inked fibers in Permount, the attained range of m.n. was 0.25 ± 0.15 compared to 0.1 ± 0.05 for a white paper.

Conclusion

Spectra of inks on paper deviate from the Beer-Lambert Law as a result of scattering and variations in the opacity of the paper in the transmission and the reflectance modes and also as a result of bronzing in the reflectance mode. Therefore, the spectra comparison based on this law (in order to eliminate concentration differences and background contributions) should be applied cautiously, especially when spectra of the compared inks may be very similar. In such cases it is preferable, when it is possible, to use distinct spectral features to discriminate between the inks.

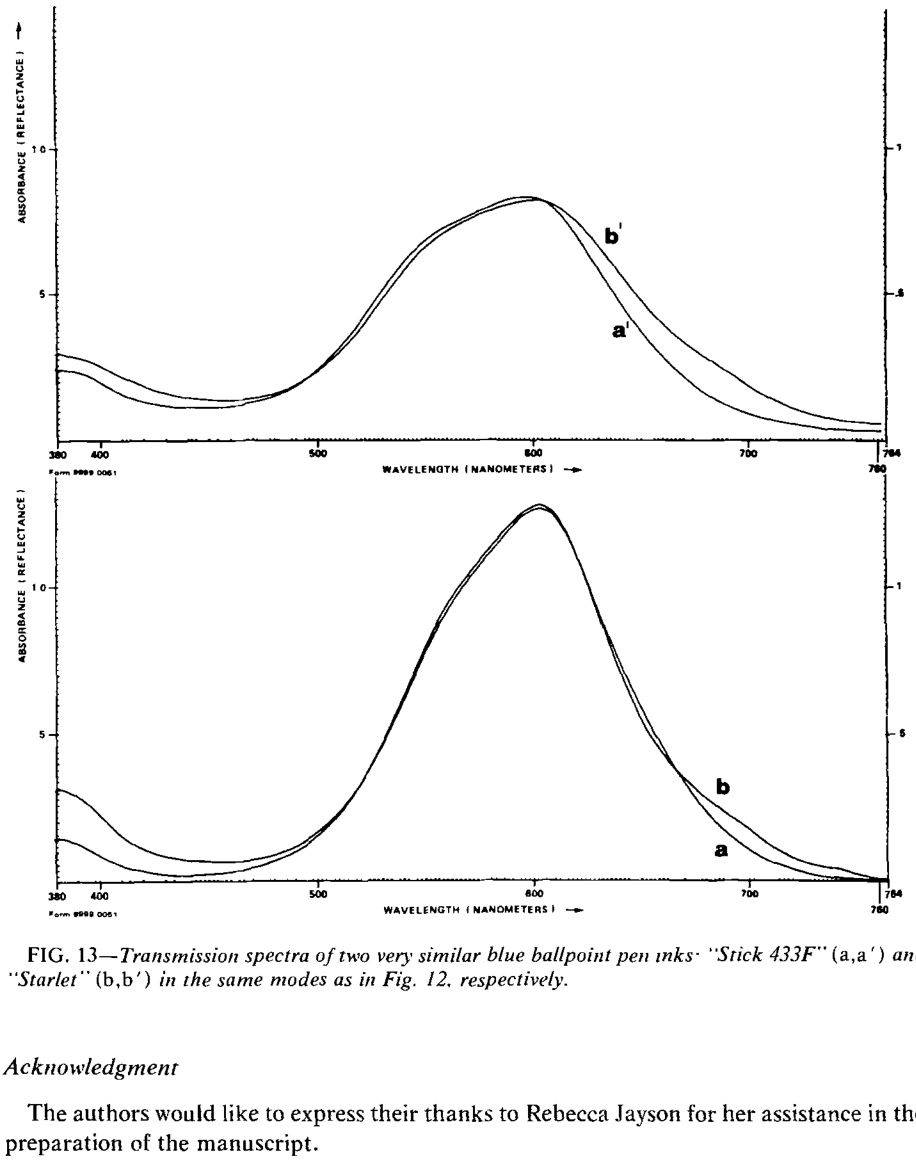

FIG. 13—*Transmission spectra of two very similar blue ballpoint pen inks· "Stick 433F"* (a,a') *and "Starlet"* (b,b') *in the same modes as in Fig. 12, respectively.*

Acknowledgment

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References

[*1*] Laing, D. K., Dudley, R. J., and Isaacs, M. D. J., "Colorimetric Measurements on Small Paint Fragments Using Microspectrophotometry," *Forensic Science International,* Vol. 16, 1980, pp. 159-171.

[*2*] Laing, D. K. and Isaacs, M. D. J., "The Comparison of Nanogram Quantities of Ink Using Visible Microspectrophotometry," *Journal of the Forensic Science Society,* Vol. 23, 1983, pp. 147-154.

[*3*] Hausdorff, H. H. and Coates, V. J., "Color Matching by Microspectrophotometry, A New Dimension in Document Examination," *Society of Photo-Optical Instrumentation Engineers,* Vol. 411, 1983, pp. 54-59.

[*4*] Pfefferli, P. W., "Application of Microspectrophotometry in Document Examination," *Forensic Science International,* Vol. 23, 1983, pp. 129-136.

[*5*] Totty, R. N., Ordridge, M. R., and Onion, L. J., "A Comparison of the Use of Visible Microspectrophotometry and High Performance Thin Layer Chromatography for the Discrimination of

Aqueous Inks Used in Porous Tip and Roller Ball Pens." *Forensic Science International*, Vol. 28, 1985, pp. 137–144.
[6] Olson, L. A., "Color Comparison in Questioned Document Examination Using Microspectrophotometry," *Journal of Forensic Sciences*, Vol. 31, No. 4, Oct. 1986, pp. 133–134.
[7] Kortum, G., *Reflectance Spectroscopy*, Springer-Verlag, New York, 1969.
[8] Wendlant, W. W. and Hecht. H. G., *Reflectance Spectroscopy*, John Wiley and Sons, New York, 1966.
[9] Jenkins, A. J. and White, H. E., *Fundamentals of Optics*, McGraw-Hill Inc., 4th ed., 1976.
[10] Messerschmidt, R. G., "Optical Properties of the Sample/Apparatus Interface in FT-IR Spectrometry," *Spectroscopy*, Vol. 1. No. 2, 1986, pp. 16–20.
[11] Suzuki, E. M. and Gresham, W. R., "Forensic Science Applications of Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS): I. Principles, Sampling Methods, and Advantages," *Journal of Forensic Sciences*, Vol. 31, No. 3, July 1986, pp. 931–952.
[12] Bauman, R. P., *Absorption Spectroscopy*, John Wiley and Sons, New York, 1962.
[13] Braun, J. H., "Bronzing of Phthalocyanine Pigments," *Official Digest*, Vol. 37, Dec. 1965, pp. 1623–1639.
[14] Wright, W. D., *The Measurement of Colour*, Adam Hilger, London, 1969, pp. 281–282.
[15] Nowicki, J. and Patten, R., "Examination of U.S. Automotive Paints: I. Make and Model Determination of Hit-and-Run Vehicles by Reflectance Microspectrophotometry," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986. pp. 464–470.
[16] Springer, E. and Zeichner, A., "The Characterization of Small Quantities of Luminescent Invisible Detection Materials Using the Docuspec TM/1 Microspectrophotometer," *Journal of Forensic Sciences*, Vol. 32, No. 1, Jan. 1987, pp. 248–253.
[17] McCrone, W. C. and Delly, J. G., *The Particle Atlas*, 2nd ed., Vol. 2, Ann Arbor Science Publishers, Inc., Ann Arbor, MI, 1973, p. 308.
[18] Gerhart, J. F. and Ward, D. C. "Paper Match Comparisons by Submersion," *Journal of Forensic Sciences*, Vol. 31, No. 4, Oct. 1986, pp. 1450–1454.

Address requests for reprints or additional information to
Dr. Arie Zeichner
Toolmarks and Materials Laboratory
Division of Criminal Identification
Israel Police Headquarters
Jerusalem, 91906 Israel