

especially with the emergence of new pen types and formulations.

Materials and Methods

Sample preparation

Twenty blue pens, consisting of ten ballpoint pens (labelled as BP) and ten gel pens (labelled as GP) were purchased from the local market. Note that the types of pen were classified according to the label on each pen, as shown in Table 1.

This study was carried out at the Questioned Document Examination Laboratory of Department of Chemistry Malaysia and the Forensic Laboratory of Royal Malaysian Police College, Kuala Lumpur. Before writing, each pen was tested by gently scribbling on a piece of paper to ensure it was functioning before sampling. Then, using each pen, a straight horizontal line of 1 mm thickness was drawn on a piece of white A4 paper (PaperOne™, Copier 70 gsm, Singapore). On a separate piece of paper, the words “Blue Pen Inks” were written using each pen by the same person to ensure standardised writing. The written samples were properly labelled for traceability. Note that the papers used were of the same type from the same rim. Prior to examination and analysis, samples were kept in a hardcover envelope in between two pieces of white A4 papers to avoid any possible two-way smudging of ink materials.

Chemicals and materials

Analytical grade methanol, ethyl acetate, ethanol, n-butanol, and 2 M hydrochloric acid (HCl) were purchased from Sigma-Aldrich (St. Louis, MO). Silica gel 60 F254 TLC plates (20 cm x 20 cm) from Merck (Kenilworth, NJ) and developing chambers were used to carry out TLC analysis.

Microscopic examination

Ink samples written on paper were firstly examined under a Leica MZ16 stereomicroscope (Leica Microsystems, Leica MZ16, Heerbrugg, Switzerland) to investigate the general morphological characteristics of pen ink deposited on the paper. The TL RC 1 external cold light source (Leica Microsystems, Heerbrugg, Switzerland) was set at 2650 K and the

written ink lines were studied under the magnification of 16×. Images of ink samples were then captured using Leica FC 290 digital camera (Leica Microsystems, Heerbrugg, Switzerland) attached to the stereomicroscope and saved as TIFF files. The microscopic system was supported by Leica Application Suite software (Leica Microsystems, Heerbrugg, Switzerland).

Video spectral comparator examination

A VSC-6000 video spectral comparator (Foster + Freeman, Worcestershire, United Kingdom) was used to examine the written ink lines and to identify differences in inks. The VSC was connected to a digital camera and a high-resolution grating spectrometer integrated with various light sources and optical filters. Written ink samples were viewed by multispectral light sources from ultraviolet to infrared regions. Barrier filters with wavelengths ranging from 400-730 nm were used to investigate the luminescence properties of ink samples. All digital images were captured, and any luminescence characteristic of inks was recorded.

Attenuated total reflectance-Fourier transform infrared spectroscopy and principal component analysis

Infrared spectra of ink samples were also obtained using Bruker FTIR Tensor 27 (Bruker Corporation, Billerica, MA) equipped with a single reflection zinc selenide attenuated total reflectance (ATR) sampling accessory. Absorbance was measured in the wavelength ranging from 600 to 4000 cm^{-1} while the resolution was set at 4 cm^{-1} . Sixteen scans were performed for each sample. OPUS 7.0.122 software (Bruker Corporation, Billerica, MA) was utilised for spectra acquisition.

Each written ink sample on paper was positioned in contact with ATR on the crystal plate at controlled ambient temperature ($\approx 25^\circ\text{C}$). Before scanning each sample, a reference background spectrum was taken at a spot close to the written line. The spectrum of each sample was taken in duplicates, compared, and evaluated. All the absorbance values of the spectrum were recorded as data points and saved to Microsoft Excel® (Redmond, WA). The data points of regions