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Chemical archaeology with historical museum samples of mauveine†

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Samples (both in powder and fabrics) from the Science Museum (ScM), Chandler Museum (CM), Museum of Science and Industry (MSIM), Deutsches Museum (Caro), and Bradford Colour Experience Museum (BcM) were analysed by HPLC-DAD, HPLC-DAD-MS, and UHPLC-HRMS to address two key aspects in the history of Perkin's mauveine: (i) the common origin of the Bradford and some of the Science Museum samples and (ii) the presence of *o*- and *p*-toluidine (and the absence of *tert*-butyl-*p*-toluidine) in the various museum samples. Regarding (i), the data show that the Bradford samples have the same origin as some of the samples from the Science Museum (London) and the Chandler Museum (New York). As the Chandler Museum sample was acquired from Perkin to mark the anniversary of the synthesis of mauveine, it can be concluded that all Bradford Museum samples also originate from Perkin, either directly or *via* his synthetic procedure. Regarding (ii), the findings confirm that the original synthetic, patented mauveine product was obtained from impure aniline containing both *o*- and *p*-toluidine, with no evidence of the presence of *tert*-butyl-*p*-toluidine.

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Introduction

Perkin's iconic mauve, or mauveine, has attracted considerable attention as a landmark in the history of chemistry.[1–14] First synthesized and patented by William Henry Perkin in 1856,[15,16] mauveine became the first commercially successful synthetic organic dye, initially marketed for silk dyeing, and later used also in the dyeing of postage stamps.[8,11]

Perkin is known to have first synthesised mauveine on 23 March 1856 and patented it later that year. The dye was subsequently produced on a modest industrial scale at Greenford, Middlesex. Perkin's synthetic approach to the mauve dye and its mauveine salts was driven by three main objectives: producing a commercially viable dye; isolating a pure compound suitable for structural characterization; and securing patent protection. For the dye to be suitable for textile applications, the chromophore needed to exhibit an appealing colour and be resistant to light, environmental exposure and washing. Mauveine met all these requirements, which contributed to its success as a dye.[17,18]

In December 1857, the first batch of Perkin's mauveine was used by the silk dyers Thomas Keith & Sons in Bethnal Green—the first dyehouse to adopt it on an industrial scale. The dye's high affinity for silk contributed to heterogeneity in the dyeing process. Perkin was also the first to introduce the method of dyeing silk in a soap bath, which ensured more uniform and stable colours. Mauveine is therefore considered a landmark in the history of chemistry and the dye industry, as it was the first fully synthetic organic dye to achieve widespread commercial success. Its maximum production was reached in 1862, and ceased in 1873.[19–21]

The so-called original mauveine, its first synthetic process and its presence in various museum samples—whether in powder form, textiles or stamps—have been studied over the last two decades,[1–4,11,18–27] long after its original synthesis in 1856.[28]

In 2007, new mauveine chemical structures were identified in historical museum samples.[3] Perkin's mauveine, analysed from museum samples, consists of more than 13 different compounds, primarily mauveine A and B, as shown in Scheme 1.[4,11]

Perkin's modification to the dye's production methods led to its commercial availability as sulphate and later acetate salts.[4] Heinrich Caro introduced a new patent-free mauveine synthesis using copper salts and with pseudo-mauveine as the major purple dye.[8,11,21]

The mauveine A/B ratio, defined as the ratio between the sum of all C26 and all C27 compound percentages, has since been used as a tracer to differentiate the synthetic routes of various mauveine samples produced by Perkin and the dominance of pseudo-mauveine in Caro's synthetic procedure.[11,22]

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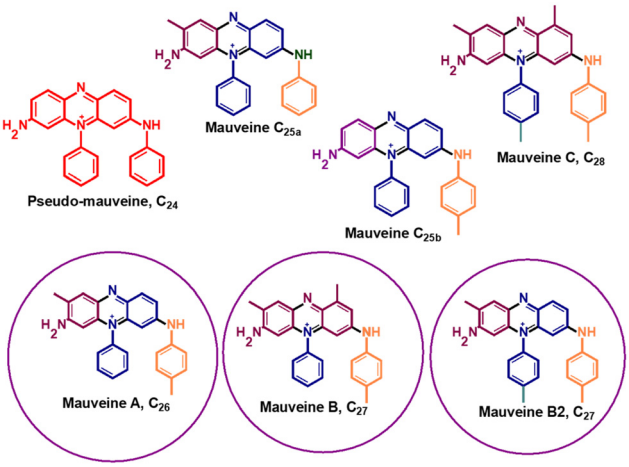

Scheme 1 Molecular structures of the known mauveine dyes found in historic museum samples according to ref. 3, 4 and 24. As will be discussed in this work, mauveine A and B are the dominant compounds in the museum samples (from Perkin synthesis), whereas pseudomauveine indicates a synthesis from Caro. In one of the Bradford Museum fabrics, mauveine C25 is the dominant compound.

The molecular structures of the mauveine dyes (Scheme 1) show that the various mauveine dyes are made up of a combination of aniline and (*o*- and *p*-) toluidine. Therefore, if Perkin had used pure aniline, his experiment would have been unsuccessful. With this in mind, Cova *et al*. used a validated modern synthesis to demonstrate that pseudo-mauveine (C24) and a series of methylated derivatives—mono- (C25), di- (C26), tri- (C27) and tetra- (C28)—can be obtained from aniline, *o*-toluidine and *p*-toluidine as starting materials.[22,29]

The ratio of *o*- to *p*-toluidine influences the formation of distinct isomeric distributions.[22] In this context, in the present work, several historical mauveine samples were examined using HPLC-DAD-MS analysis to detect traces of toluidine (*m*/*z* 107.90), which would support the use of Perkin's synthetic procedure. In addition to the samples previously analysed from four museums, we have included specimens from the Bradford Colour Experience Museum. This increases the number of museum samples examined, allowing us to gain a clearer understanding of whether these samples share a common or distinct origin.

Experimental

Historical samples

The historical samples analysed in this study, including those from the Colour Experience Museum (Bradford), see picture in Fig. SI1,† were compared with samples from the Science Museum (London), Chandler Museum (Columbia University, New York), Museum of Science and Industry (Manchester), and Caro (Deutsches Museum, Munich, Germany), as described in previous studies.[4] For fibre analysis, the extraction procedure followed the method described in ref. 4.

HPLC-DAD analysis

The distribution of the several mauveine chromophores was established in an Elite Lachrom HPLC-DAD system with an L-2455 Diode Array Detector, L-2300 Column Oven (RP-18 end capped column), L-2130 Pump and L-2200 Auto Sampler. All the samples were dissolved in methanol. A solvent gradient method was performed with acidic water (formic acid, 0.1% v/v) (A) and methanol (B), with a flow rate of 1.5 mL min^{-1}: 0–8 min: 85% A/15% B; 8–15 min: 50% A/50% B; 15–20 min: 40% A/60% B; 20–25 min: 30% A/70% B; 25–30 min: 25% A/75% B; 30–35 min: 20% A/80% B; 35–40 min: 15% A/85% B; 40–55 min: 10% A/90% B; 55–65 min: 50% A/50% B; 65–75 min: 85% A/15% B, with an oven temperature of 22 °C. The HPLC-DAD chromatograms were acquired at a wavelength of 550 nm.

The peak areas, including their percentages and retention times of each chromophore were obtained with the chromatographic program EZChrom Elite. All measurements were performed in triplicate, and the results were found to be reproducible for all samples.

HCA analysis

The chromatographic data (retention times and integrated areas) were analysed using Hierarchical Cluster Analysis (HCA) with the statistical package SIMCA 16.[30,31] The data set includes information for all samples (ScM, CM, MSiM, Caro, BcM), including their retention times and area ratios.

HPLC-DAD-MS analysis

Aliquots of 10 μL of methanolic extracts of the mauveine samples (ScM, CM and MSIM) were analysed on a Dionex Ultimate 3000SD system with a diode array detector coupled in-line to an LCQ Fleet ion trap mass spectrometer (Thermo Scientific, Waltham, MA, USA). Separations were carried out with a Kinetex C18 100 Å (150 × 2.1 mm, 5 μm, Phenomenex, USA) at 40 °C, using a flow rate of 0.3 mL min^{-1}. The mobile phase consisted of 0.1% formic acid (v/v) in water (eluent A) and in acetonitrile (eluent B), and the elution gradient was as follows: 0–1 min, linear gradient to 0% B; 1–5 min, linear gradient to 7% B; 5–18 min, linear gradient to 100% B; 18–22 min, isocratic 100% B; and then the column was re-equilibrated with 0% B for 7 min. The mass spectrometer was operated in the ESI positive mode, and the optimized parameters were as follows: ion spray voltage, +4.5 kV; capillary voltage, 16 V; tube lens offset, −70 V; sheath gas (N_2), 40 arbitrary units; auxiliary gas (N_2), 20 arbitrary units; and capillary temperature, 300 °C. Spectra typically correspond to the average of 20–35 scans and were recorded in the range between 100–1000 Da. Data acquisition and processing were performed using the software Thermo XCalibur 2.2. SP1.

UHPLC-HRMS analysis

Aliquots of 2 μL of the methanolic mauveine extracts (ScM, MSIM, CM and BcM) were also analysed using a UHPLC Elute system coupled to a QqTOF Impact II high-resolution mass

spectrometer with an ESI ionization source (Bruker Daltonics, Billerica, MA, USA). The samples were analysed in ESI positive mode, and the optimized spectrometer parameters were as follows: end plate offset, 500 V; capillary voltage, +4.5 kV; nebulizing gas (N_2), 2.8 bars; dry gas (N_2), 8 L min^{-1}; and dry temperature, 200 °C. Calibration of the TOF analyser was performed with a solution of sodium formate 10 mM, introduced into the ion source through a six-port valve with a 20 µL loop, at the beginning of each analysis. Mass spectra were acquired in a full scan mode, in the mass range between 100 and 100 *m*/*z*, and an acquisition speed of 3 Hz. The configuration and control of the LC/MS interface was performed using Compass Hystar software, and the acquired MS data were processed using Data Analysis (versions 4.5 and 5.1, Bruker Daltonics). Chromatographic separation was achieved with a C18 column Kinetex 100 Å (100 × 3.0 mm; particle size 2.6 µm; Phenomenex, USA), using an elution gradient of 0.1% v/v formic acid in water (phase A) and in acetonitrile (phase B) and a flow rate of 550 µL min^{-1}. The elution conditions were as follows: 0–1 min, isocratic at 5% B; 1–11 min, linear gradient up to 100 B; 11–15 min, isocratic at 100% B; 15–17.5 min, linear gradient up to 5% B followed by 4 min of re-equilibration of the column. The chromatographic column and sampler were maintained at a temperature of 50 °C and 8 °C, respectively.

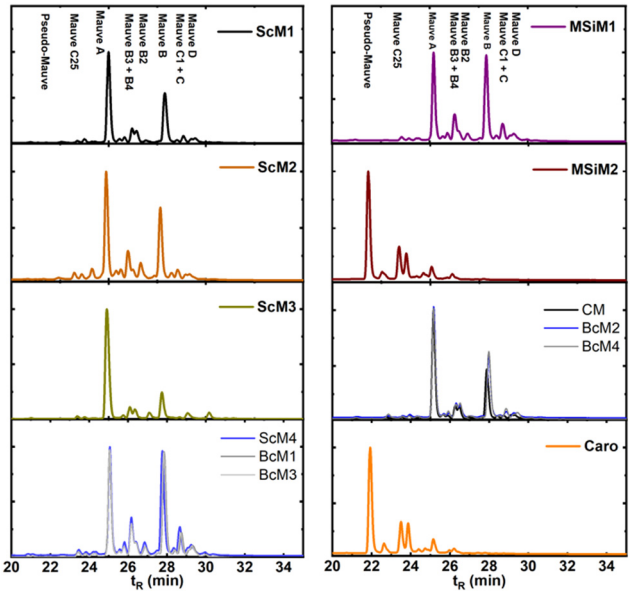

Fig. 1 HPLC-DAD chromatograms obtained at 550 nm for the Science Museum (ScM1–4), Manchester Science and Industry Museum (MSIM1–2), Chandler Museum (CM), Deutsches Museum (Caro) and Bradford Museum (BcM1–BcM4) samples. Main chromophores: mauveine A at t_R = 25.05 min and mauveine B at t_R = 27.76 min.

Results and discussion

To increase the number of museum samples analysed, powder and silk fabric samples from the Bradford Colour Experience Museum (see the ESI† for details) were analysed by HPLC-DAD and compared with samples from other museums (Science Museum, ScM; Chandler Museum, CM; Museum of Science and Industry, MSIM; and Deutsches Museum, Caro; and Perth Museum). The vestigial presence of *o*- and *p*-toluidine was also investigated in historical museum samples previously studied.[3,4]

Comparison of Bradford Colour Experience Museum samples with other museum specimens

To directly compare the Bradford Colour Experience Museum with previously studied samples, samples from the Science Museum (ScM1–ScM4), the Chandler Museum (CM), the Museum of Science and Industry (MSIM1 and MSIM2) and the Deutsches Museum (Caro) were analyzed under the same experimental conditions using HPLC-DAD (Fig. SI1†). The data in Fig. 1 show that the HPLC-DAD chromatograms of the museum samples here obtained agree with previous results,[4,11] thus validating the comparative analysis of the Bradford Colour Experience Museum (BcM1–BcM4) samples. A closer examination shows that mauveine A is the predominant compound in the ScM1, ScM3, CM, BcM2 and BcM4 samples. In contrast, mauveine B (considered as the sum of mauveine B, B2, B3 and B4) is the major component in ScM2, ScM4, MSIM1 BcM1 and BcM3 (see Table SI1†). In the MSIM2 sample, pseudo-mauveine accounts for more than 50% of the mixture, with smaller amounts of mauveine A, while the presence of mauveine B is minimal (including B, B2/B3/B4 totalling ~2%), Table SI1.† In the Deutsches Museum (Caro) sample, pseudo-mauveine is again the most abundant compound, accounting for more than 50% of the composition, with C25 (a and b) compounds accounting for more than 30%.

The newly analysed samples include four mauve powders – BcM1, BcM2, BcM3 and BcM4 – corresponding to different bottles labelled "Acetate of Mauve", "Pure Acetate", "Mauve Acetate" and "Mauveine" respectively (see Table 1 and Table SI1† for data and a closer view of the original source in Fig. SI1†). In addition, two silk fabric samples, BcM_F1 and BcM_F2, were included. These silk fabric samples, which have distinct yarn characteristics, are believed to have been dyed by Perkin himself. Following the previous methodology, the Bradford Museum samples (BcM1–BcM4) were analysed by HPLC-DAD, confirming that none of the samples contained traces of pseudo-mauveine. Fig. 1 shows the HPLC-DAD chromatograms of ScM4, BcM1 and BcM3, which are highly similar. Similar chromatographic profiles are also observed for ScM1 and ScM4, and for BcM2 and BcM4, see also Table SI1.† The ratio of mauveine C26 (mauveine A) to mauveine B (including all C27 isomers), *i.e.*, the mauveine A to mauveine B ratio, is indicative of the synthetic procedure originally employed by Perkin, as previously described,[4] and is compared with previous studies[4,11] in Tables SI1 and SI2.† Tables 1 and SI1† (for comparison with the other museum samples) show that the main chromophores identified in the Bradford Museum samples BcM1 to BcM4 are mauveine A and mau-

Table 1 Relative percentage area of the main chromophores in the mauveine samples from the Bradford Museum.

	Pseudo-mauve	Mauve C25 (a, b)	Mauve A (C26)	Mauve C27 (B2/B3/B4)	Mauve B	Mauve C1	Mauve C	Ratio[a]
BcM1	—	1.31	33.30	18.87	34.81	6.82	4.90	0.62
BcM2	—	0.50	51.14	12.23	30.27	2.12	3.74	1.20
BcM3	—	1.33	33.24	19.19	34.67	6.83	4.74	0.62
BcM4	—	2.80	46.53	12.99	30.17	3.70	3.81	1.08

[a] Ratio of the Mauve C26/Mauve (all C27) obtained in all the investigated museum samples. See Table SI1† for the other museum samples.

veine B (considering all C27 compounds as mauveine B). The highest area percentage of mauveine B is exhibited in BcM1 and BcM3, whereas in BcM2 and BcM4, higher proportions of mauveine A are present.

Additionally, UHPLC-ESI(+)/HRMS analysis (Fig. 2) was performed for all the samples including the new Bradford Museum (BcM1–4) and further compared with the previously analysed mauveine museum samples.[4] As with the data in Fig. 1 (and Table SI1†) there is a clear match of BcM1 and BcM3 with the ScM4 sample and of BcM2 and BcM4 with the CM sample (and with ScM1).

In the case of the previously analysed museum samples,[4] the profile of the different mauveine dyes shows peaks at *m*/*z* 377 (mauveine C25), *m*/*z* 391 (mauveine A), *m*/*z* 405 (mauveine B, B2, B3 and B4), *m*/*z* 419 (mauveine C) and *m*/*z* 433 (mauveine D). Nevertheless, it is interesting to note that for *m*/*z* 391, attributed to mauveine A (at a t_R of 6.45 min), there are two peaks (mirroring two possible isomers), whereas for mauveine B (all C27 mauveines), there are five peaks, B, B2, B3, B4 and one that coelutes with the additional mauveine A (at a t_R of 6 min). However, these two additional isomers of mauve A and mauve B are present in lower amounts.

Fig. 2 shows the ion chromatograms for the mauveine samples ScM4, BcM1 and BcM3, which clearly identify the two possible isomers. Coupling UHPLC with high-resolution mass spectrometry offers additional benefits in terms of selectivity and sensitivity when analysing complex matrices. However, further detailed experiments would be needed to confirm the presence of additional isomers in mauveine dyes.

Consequently, to properly integrate the peaks, the data in Tables 1, S1 and S2† were obtained with the HPLC-DAD data where the different mauveine dyes were previously identified with mauveine A at a t_R of 25.05 min and mauveine B at a t_R of 27.76 min (and the additional B3 + B4 at a t_R of 26.2 min and mauveine B2 at a t_R of 26.96 min) under the present experimental conditions. These differences in the relative proportions of mauveine A and B support the existence of two distinct synthetic processes for Perkin's mauveine.[4,15] The first synthetic route favours a higher percentage of mauveine A, while the second favours a higher percentage of mauveine B.[4,15] Accordingly, BcM2 and BcM4 appear to originate from the first synthetic route in which mauveine A was dominant, whereas BcM1 and BcM3 correspond to Perkin's second (improved) synthetic route.[4] Fig. 1 and Table SI1† also show that BcM1 and BcM3 are very similar to ScM4 and MSIM1.

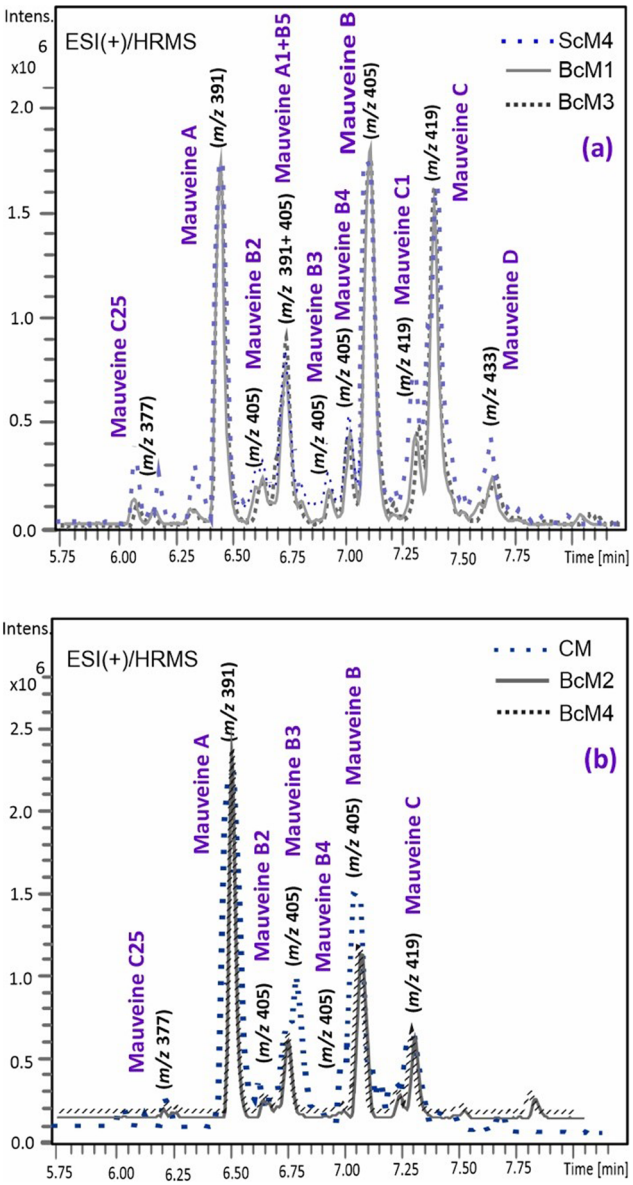

Fig. 2 UHPLC-HRMS total ion chromatograms obtained in the ESI positive mode for (a) ScM4, BcM1, and BcM3 and (b) CM, BcM2, and BcM4, and (b) mauveine extracts.

This similarity is evident in the distribution of the different mauveine dyes, particularly in the ratio of mauveine A/B, as shown in Tables SI1 and SI2.† In contrast, BcM2 and BcM4 appear to share a common origin with ScM1 and CM (see the

mauve A/mauve B ratio of 1.1 to 1.2 in Table SI1†). Overall, these results suggest that samples BcM1 and BcM3 from the Bradford Colour Experience Museum are identical to those from the Science Museum (ScM2 and ScM4) and the Museum of Science and Industry in Manchester (MSIM1), while samples BcM2 and BcM4 correspond to those from the Science Museum (ScM2) and the Chandler Museum (CM).

To fully identify the purple chromophores present in the total ion chromatograms (TIC), the ion chromatograms were obtained for each cationic species under analysis (Fig. SI2†).

Hierarchical Cluster Analysis was performed to define the data structure in Tables 1 and SI1,† allowing the data to be classified into clusters.[22] The resulting dendrogram, based on the relative percentages of the different mauve compounds identified in Tables 1 and SI1,† categorizes the samples into four distinct groups and is presented in Fig. SI3.† There is a clear distinction between group 1 and groups 2, 3 and 4. Group 1 (consisting of MSIM2 and Caro) is clearly separated from the other groups. This separation is mainly due to the presence of pseudomauveine in group 1 – a component absent in group 2 – and the presence of mauveine C25, with only residual amounts of mauveine A and B. Although groups 2, 3, and 4 share similarities due to the presence of both mauveine A and B, they also exhibit distinct differences. Specifically, group 2 consists only of the ScM3 sample; group 3 includes ScM1, CM, BcM2 and BcM4; and group 4 includes ScM2, ScM4, MSIM1, BcM1 and BcM3. This distinction is primarily based on the relative proportions of mauveine A and B in the samples. Sample ScM3 has the highest ratio (2.31), indicating a significantly greater contribution of mauveine A relative to mauveine B (again considering all C27 compounds). In contrast, groups 3 and 4 show lower ratios of about 1.14 and 0.60, respectively. It is noteworthy that the samples in group 4 contain a higher proportion of mauveine B in relation to mauveine A.

Analysis of the Bradford Museum fabric samples

Further analysis of the Bradford fabrics (BcM_F1 and BcM_F2) was carried out and compared with the previously analysed fabrics from the Science Museum (ScMF1 to ScMF6),[4] as shown in Table SI3.† The textile analysis was initially carried out using the UV-VIS spectroscopy technique. To achieve a more detailed characterization, HPLC-DAD analysis of the extracted colouring material was performed (Fig. 3). Previous work on fabrics dyed with mauveine[4] divides the textiles into two groups: the first is characterized by mauveine A and B as the primary chromophores, with small amounts of mauveine C25; the second with mauveine A as the main chromophore, accompanied by mauveine C25.

In Table SI3,† BcM_F1 and BcM_F2 show clear differences in chromophore composition. BcM_F1 has the highest levels of mauveine C25, while containing only minimal amounts of mauveine A and B. In contrast, BcM_F2 follows the expected composition of Perkin's synthetic dye, with mauveine A and B as the major chromophores. It can be concluded that BcM_F2 has the same origin as ScMF1, while BcM_F1, although closer to ScMF5 and ScMF6, contains a much higher amount of mauveine C25.

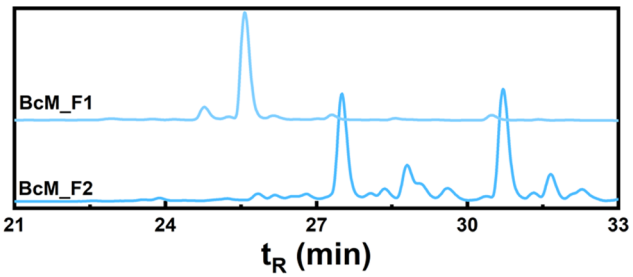

Fig. 3 HPLC-DAD chromatograms obtained at 555 nm for the Bradford Museum fabric samples (BcM_F1 and BcM_F2); main chromophores: mauveine C25 at a t_R of 25.57 min, mauveine A at a t_R of 27.50 min and mauveine B at a t_R of 30.70 min.

Indeed, mauveine C25 is the predominant chromophore in BcM_F1, accounting for over 93% of its composition. The closest matches to BcM_F1 are the MSIM2 and Deutsches Museum (Caro) samples, which contain the highest relative amounts of C25 – approximately 34% (see Tables 1 and SI1†). However, these samples do not correspond to BcM_F1, as they lack pseudo-mauveine, the distinguishing marker of Caro's mauveine. This significant difference supports the idea that BcM_F1 was dyed using a different synthetic method.

Evidence for Perkin's synthetic procedure from the detection of toluidine traces by HPLC-DAD-MS

Previous chemical analyses of various museum samples indicated that the so-called original mauveine sample from the Science Museum could not have been the first dye produced by Perkin.[3,4] The only dated sample linked to Perkin's original mauveine synthesis, or dyed using the original process, was identified on a fabric from a woollen shawl exhibited at the 1862 Exhibition, a period when mauve was sold as an "amorphous body".[3,4] However, this was later questioned by others, who argued, based on a letter from F. Mollwo Perkin (the son of William Perkin), that the Science Museum sample (ScM1) might indeed be the original mauveine. This argument was primarily supported by the label that read "Original Mauveine prepared by Sir William Perkin in 1856", suggesting that it may not have been made by the patented method, but rather by an alternative synthesis that replaced *p*-toluidine with *tert*-butyl-*p*-toluidine hydrochloride.[32–34]

A critical analysis of the label and the contents of the bottle had previously been carried out by P. J. T. Morris.[2] It is worth noting that the sample from the bottle in the Science Museum was analysed in 2008 and designated ScM1 (Fig. 1 and Tables SI1 and SI2†).[4] This ScM1 sample is sometimes referred to as "original mauveine" due to its label; however, subsequent analyses of this and other museum samples[4] and of postage stamps containing mauveine[8,11] showed that it could not be considered the result of the original synthetic procedure. Indeed, in addition to the chemical analysis of the various mauveine compounds by HPLC-DAD-MS, the analysis of the

historic mauveine sample (ScM1) by HPLC anion exchange chromatography (HPLC-AEC) of the counter-ions in the mauveine salts revealed the presence of 97% acetate salt and 0% sulfate salt.[4] As Perkin himself wrote, in the original synthetic attempts "aniline was selected, and its sulphate was treated with potassium dichromate"[35] and that mauve was first sold as an "amorphous body", in the form of the sulphate salt. After this initial process, the mauve dye was then manufactured in a second optimized process and sold as the acetate salt of mauveine. This happened because the first salt, the mauveine sulphate, was not sufficiently soluble to be used as a dye and as Perkin stated it was "unsuitable for the dyer".[35] "(...) this crystalline colouring matter in alcohol, (...) consisted of what we now know as the sulphate of mauveine. As this salt is difficultly soluble in alcohol, and unsuitable for the dyer, it was boiled in alcohol with potassic acetate and thus converted into the soluble acetate of mauveine".[15]

Therefore, the original mauveine, whether referring to the sample or the synthetic procedure, must contain the sulphate counter-ion in the mauveine salts. In the 2008 study made in different museum samples of mauveine,[4] the dominant counter-ion was acetate (ScM1, ScM4, MSIM1, MSIM2 and CM) except for ScM2 and ScM3 where sulphate was dominant.[4] However, despite this investigation into the chemical composition of mauveine samples, a more precise approach for tracing the original source materials involves detecting residual traces of *p*-toluidine and *o*-toluidine—the impurities present in aniline during Perkin's synthesis.[15,22,24] In his own words: "It was soon found, however, that by using an aniline containing much larger quantities of toluidine, a colouring matter giving redder shades of purple was produced".[15] Particular attention is drawn to the unusual claim that *p*-toluidine was replaced with *tert*-butyl-*p*-toluidine in an alternative method of synthesis.[33] This substitution is considered highly unlikely and contradicts established practice in the historical chemical methodology of mauveine.[7,22,29]

To determine the presence of residual traces of toluidine, historical mauveine samples were analyzed using HPLC-DAD-MS. The absorption spectra in the chromatograms were obtained in the UV region (λ = 260 nm) where toluidine exhibits absorption. Both *o*- and *p*-toluidine have a molecular weight of 107.16 g mol^{-1}, while *N*-*tert*-butyl-*p*-toluidine has a molecular weight of 163.26 g mol^{-1}. HPLC-DAD-MS analyses of the historical mauveine samples (ScM1–4, MIMS1, MIMS2 and CM) were performed prior to testing the *ortho*- and *para*-toluidine samples to confirm that there was no contamination from the toluidine samples in either the chromatographic column or the mass spectrometer. T*ert*-butyl toluidine is expected to have a t_R similar to that of the two toluidine samples analysed, with a slightly longer retention time. The HPLC-DAD-MS results for the background (water matrix), *o*-toluidine and *p*-toluidine samples are given in the ESI (Fig. SI3 and SI4†).

The HPLC-DAD-MS analysis of the mauveine sample ScM1 is shown in Fig. 4, which includes (a) the HPLC-DAD chromatogram recorded at 260 nm; (a′) the total ion chromatogram (TIC) obtained in ESI positive ion mode (dashed line), together with the extracted ion chromatograms (EIC) for the cationic species of mauveine C25 (*m*/*z* 377.32), mauveine A (*m*/*z* 391.37), mauveine B (*m*/*z* 405.32), mauveine C (*m*/*z* 419.36) and mauveine D (*m*/*z* 433.35); (b) the extracted ion chromatogram in ESI positive mode for *m*/*z* 107.90; (b′) the MS spectrum obtained at the top of the peak at t_R = 5.28 min corresponding to the protonated molecule of toluidine (*m*/*z* 107.90) and (b″) the MS^2 spectrum of the precursor ion *m*/*z* 107.90 assigned to a toluidine structure; (c) the extracted ion chromatogram in ESI positive mode for *m*/*z* 164.14 in which no measurable signal was observed for the protonated molecule of *tert*-butyl-*p*-toluidine.

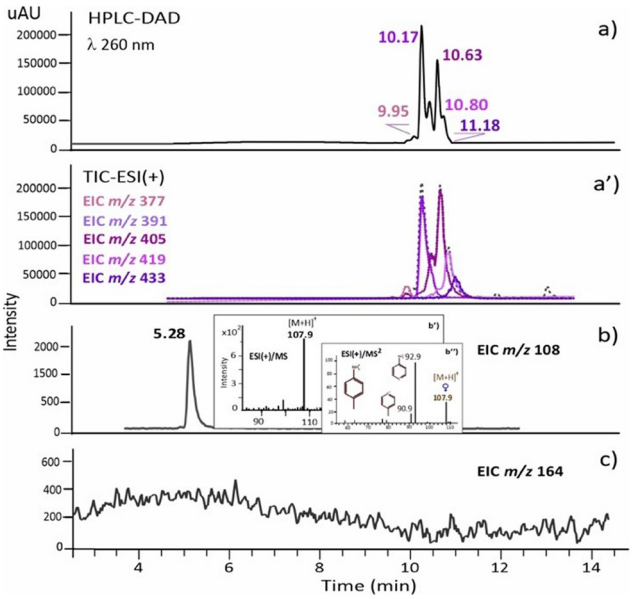

Fig. 4 HPLC-DAD-MS analysis of the mauveine sample ScM1. (a) HPLC-DAD chromatogram at 260 nm; (a′) total ion chromatogram obtained in the ESI positive mode, and extracted ion chromatograms for the major purple chromophores present in the sample; (b) extracted ion chromatogram for the protonated molecule at *m*/*z* 107.90; (b′) (inset in b) MS spectrum obtained at the top of peak at a t_R of 5.28 min; (b″) (inset in b) MS^2 spectrum of the precursor ion *m*/*z* 107.90 attributed to the protonated molecule of toluidine; (c) extracted ion chromatogram for the protonated molecule at *m*/*z* 164.14.

The results presented in Fig. 4, particularly in panels (b) and (c), clearly indicate that ScM1 contains traces of (*o*- or *p*-) toluidine, while no evidence of *tert*-butyl-toluidine is present. The HPLC-DAD-MS results obtained for the other museum mauveine samples, ScM2–4, MIMS1 and CM (see Fig. SI5–S9 in the ESI†), exhibit a similar behaviour to that observed for sample ScM1 (Fig. 4). Sample MIMS1 (Fig. SI9†), MSIM2 (Fig. 5) and ScM4 (Fig. SI7†) contained small traces of toluidine, while the strongest toluidine signal was detected in mauveine sample ScM1 (Fig. 4). The chromatographic conditions optimised for the analysis of the mauveine samples did not allow for efficient separation of *o*- and *p*-toluidine, as shown in Fig. SI4.† Therefore, it can only be concluded that both *o*- and *p*-toluidine were used in the production of mauveine. However,

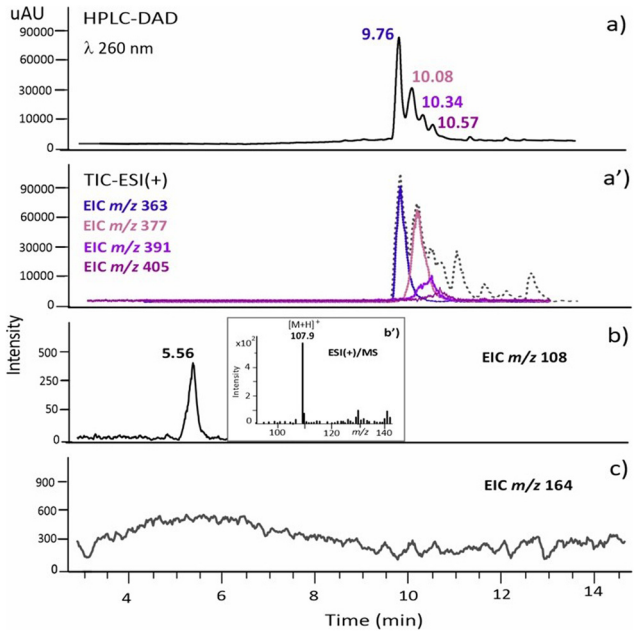

Fig. 5 HPLC-DAD-MS analysis of the mauveine sample MSIM2. (a) HPLC-DAD chromatogram at 260 nm; (a') total ion chromatogram obtained in the ESI positive mode, and extracted ion chromatograms for the major purple chromophores present in the sample; (b) extracted ion chromatogram for the protonated molecule at *m*/*z* 108; (b') (inset in b) MS spectrum obtained at the top of peak at a t_R of 5.56 min; (c) extracted ion chromatogram for the protonated molecule at *m*/*z* 164.

HPLC-DAD-MS analysis clearly showed that *tert*-butyl toluidine was not involved in the synthesis of this set of historical mauveine samples.

It is noteworthy that the MSIM2 sample exhibits a distribution of mauve dyes almost identical to that of Caro's sample.[21] Caro moved to Manchester, where he was affiliated with Roberts, Dale & Co. from 1859 to 1866. This similarity suggests that both samples have a common origin. MSIM2 contains pseudo-mauveine (*m*/*z* 363, see Fig. 5), accounting for over 58% of the mixture. The sample also contains mauve C25 (34%), as well as minor amounts of mauveine A and trace levels of mauveine B (see Fig. 1). Additionally, it shows vestigial amounts of toluidine, as previously mentioned. This suggests the presence of contaminated aniline or toluene, as the initial benzene derived from coal tar was probably contaminated with toluene. Upon nitration and subsequent reduction, this contamination yielded a complex mixture of anilines and toluidines.[7] Among Caro's many contributions during his period in Manchester was the development of a patent-free process for producing mauve.[21]

Conclusions

In summary, museum samples were analysed using HPLC-DAD, HPLC-DAD-MS and UHPLC-HRMS to investigate the common origin of the Bradford, Science Museum (London), Manchester Science and Industry Museum and Chandler Museum (Columbia University) samples and the presence of *o*- and *p*-toluidine. The *o*- and *p*-toluidine were found in trace amounts in ScM2–4, MSIM1–2 and CM, with a more intense signal of toluidine for ScM1. The findings confirm that Perkin's samples, whether produced by him directly or according to his method, originated from aniline contaminated with toluidine rather than pure aniline. No pseudo-mauveine was found in the BcM samples, which are very similar to the ScM samples. BcM1, BcM3 and BcM_F2 are very similar to ScM4, whereas BcM2 and BcM4 are more similar to CM and ScM1–3. All of these samples share the fact that they were synthesised by Perkin himself or by his synthetic method. Mauve C25 was found to be the major chromophore in BcM_F1, as the area percentage is greater than 90%, indicating that this fabric was most likely dyed with a different synthetic method.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the ESI†, and all other data will be made available upon reasonable request.

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