SHORT COMMUNICATION

The mystery of the discolored flints. New molecules turn prehistoric lithic artifacts blue

Andrea Tapparo · Gilberto Artioli · Ivana Angelini · Gabriella Favaro

Abstract Prehistoric artifacts turning blue in the store rooms of the Natural History Museum in Verona, Italy recently raised serious issues for heritage materials conservation. Our analytical investigation showed that the unusual discoloration process of the flint tools is caused by the surface presence of at least three previously unknown pigmenting molecules of the triphenylmetane dyes class: 6-(bis(2,2,4-trimethyl-1,2-dihydroquinolin-6-yl)methylene)-2,2,4-trimethyl-2,6-dihydroquinolinium and its hydrogenated derivatives 2,2,4-trimethyl-6-((2,2,4-trimethyl-1,2,3,4-tetrahydroquinolin-6-yl)(2,2,4-trimethyl-1,2-dihydroquinolin-6-yl)methylene)-2,6-dihydroquinolinium and 6-(bis(2,2,4-trimethyl-1,2,3,4-tetrahydroquinolin-6-yl)methylene)-2,2,4-trimethyl-2,6-dihydroquinolinium. The peculiar formation of the molecules is possibly catalyzed within the silica pore surface starting from a well-known rubber stabilizer 2,2,4-trimethyl-1,2-dihydroquinoline released by the plastic pads flooring the storing cabinets. The investigated reaction and its surprising blue product represent a case study of the application of modern materials science to conservation and a serious warning towards the unpredictable challenges faced in the preservation of our cultural heritage.

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Introduction

The conservation of cultural heritage collections requires a thorough understanding of the materials involved and careful control of the storage conditions [1]. For many common materials, appropriate and specific conservation protocols have been developed, so that the integrity of the objects is preserved for extended periods of time. Silica-based lithic materials such as cherts and flints, widely used in prehistory for making sharp tools, are commonly considered to be rather unreactive materials [2] posing no special problems to conservation in museum collections [3, 4]. For this reason, the surprising and unusual process of blue discoloration observed in some of the prehistoric artifacts of the archaeological collection of the Natural History Museum in Verona, Italy caused special concern in the authorities in charge of the collections and had large echoes in the national and international press [5, 6]. Soon after the discoloration process was diagnosed, upon formal request of the "Soprintendenza per i Beni Archeologici del Veneto", intensive analytical research was undertaken in order to characterize the unusual physico-chemical mechanisms causing the pigmentation of the flint tools and to develop strategies to stop and eventually revert the process. The solution to the puzzle proved to be much harder than expected and required a great deal of interdisciplinary analytical and experimental work.

Since the nearby Lessini mountains are essentially formed of Mesozoic and Caenozoic sedimentary limestone formations containing abundant fossils and flint nodules, The Natural History Museum in Verona, Northern Italy
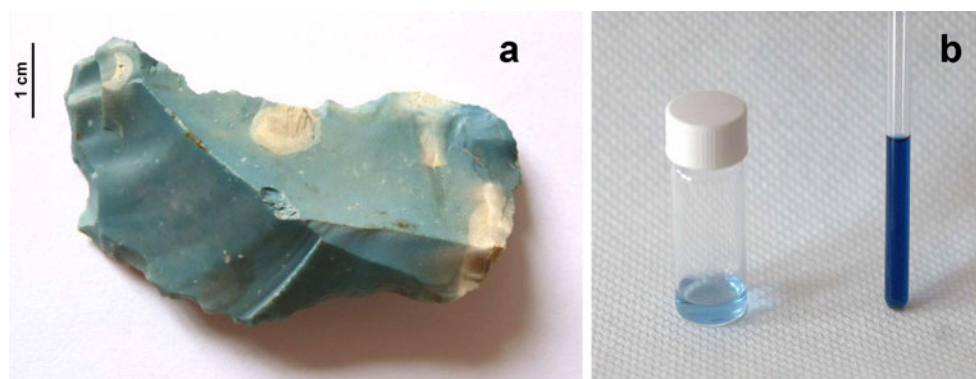
hosts among a variety of specimens of a spectacular collection of marine fossil, notably the world-famous and exceptionally preserved Eocene fish from the Bolca quarry [7, 8] and a huge amount of prehistoric lithic artifacts of different ages made of local flints [9, 10]. Due to the recent rearrangements of the collections, part of the archaeological material was moved 2 years ago from the original locations in Castel San Pietro and Palazzo Gobetti to a new storage location in the restored Arsenale buildings, a dismissed military arsenal that would become the permanent site for the archaeological section of the Museum. At that time the archaeological collection of lithic artifacts was reorganized and stored in newly prepared cabinets.

Shortly after the reorganization, it was noticed that some artifacts had changed into different shades of blue (Figure 1a) and in Spring 2010 the "Soprintendenza per i Beni Archeologici del Veneto" officially provided several discolored samples to our group for investigation, shortly before the "mystery of the blue flints" hit the national and international news [5, 6].

Materials and methods

Optical microscopy was performed on polished thin sections of the sample using a Nikon Eclipse ME600L polarizing microscope, both in transmitted and reflected light. The LC-MS-MS instrument used was an Agilent (Santa Clara, CA, USA) U-HPLC 1200 equipped with photodiode array detector and Q-TOF 6520 MS detector. The operating conditions were: kinetex C18 100×2.1, 2.6 μm core shell (Phenomenex, Torrance, CA, USA) column; eluent trifluoroacetic acid 0.1% in water/acetonitrile (ACN), gradient mode: 0–2 min at 60% ACN, from 2 to 10 min to 100% ACN, 5 min at 100% ACN, to 60% ACN in 1 min, 5 min of equilibration; flow rate 0.2 mL min^{-1}; column temperature 30 °C; injection volume 5 μL; Electro-Spray Ionization mode, positive; capillary voltage 4,000 V; nebulizer gas 35 psig; drying gas 11 L min^{-1}, 350 °C; fragmentor 175 V; collision gas nitrogen; collision voltage: 35, 50, 70, and 100 V.

Under these conditions, retention times of main blue species, Juliet Blue, Romeo Blue, and Flint Blue (Fig. 4), were 10.82, 11.13, and 11.47 min, respectively, and the following ESI-MS experimental observations were obtained (main m/z signals are marked in bold).

Juliet Blue ($C_{37}H_{42}N_3^+$, MM=528.75 gmol^{-1}) ESI-Q-TOF/MS signals: m/z [M$^+$] 528.3390 amu with main ^{13}C isotopic ions at 529.3422 and 530.3487 amu. ESI-Q-TOF/MS-MS m/z signals, precursor ion 528.339 amu, collision voltage up to 50 V: 513.317, **498.293**, and 483.268 amu; collision voltage 70 V: 498.287, **482.257**, 468.241, 453.217, and 325.169 amu; collision voltage 100 V: **481.248**, 466.225, 452.209, 323.153, 309.137, 295.122, and 157.087 amu.

Romeo Blue ($C_{37}H_{44}N_3^+$, MM=530.76 gmol^{-1}) ESI-Q-TOF/MS signals: [M$^+$] 530.3541 amu with main ^{13}C isotopic ions at 531.3575 and 532.3618 amu. ESI-Q-TOF/MS-MS signals, precursor ion 530.354 amu, collision voltage up to 50 V: 515.332, **500.306**, and 485.284 amu; collision voltage 70 V: **499.298**, 485.281, 470.256, 443.235, and 429.219 amu; collision voltage 100 V: 483.264, **468.241**, 454.226, 453.22, **442.227**, 428.211, 412.184, 400.184, 323.153, 309.138, 297.138, 285.137, 271.124, and 158.096 amu.

Flint Blue ($C_{37}H_{46}N_3^+$, MM=532.78 gmol^{-1}) ESI-Q-TOF/MS signals: [M$^+$] 532.3701 amu with main ^{13}C isotopic ions at 533.3735 and 534.3764 amu. ESI-Q-TOF/MS-MS signals, precursor ion 532.370 amu, collision voltage up to 50 V: **517.347**, 502.322, and 461.284 amu; collision voltage 70 V: **516.336**, 502319, 486.289, 461.281, 446.259, 431.235, and 405.219 amu; collision voltage 100 V: 500.302, 485.279, 470.256, 456.242, 444.242, **430.226**, 415.206, 404.212, 388.186, 311.153, 297.135, 285.138, 271.124, and 158.095 amu.

Results and discussion

The study of the unusual coloring process acting on the flints started with routine mineralogical, geochemical, and analytical techniques [2, 11]. Visible reflectance spectros-

Fig. 1 a Photograph of an altered flint displaying an amazing *blue tinge*. b The acetone extracts of the coloring molecules. The discolored flints were nicknamed "avatar flints" in the local press

copy, X-ray polycrystalline diffraction, Fourier-transform infrared and micro-Raman spectroscopies, laser ablation mass spectrometry, optical microscopy, scanning electron microcopy, and gas chromatography–mass spectrometry were applied to the surface and the sectioned parts of the flint samples, in the attempt to identify the coloring mechanism. Surprisingly, all preliminary chemical and spectroscopic analyses failed to detect any coloring ion, such as copper and cobalt, or common organic dying molecules or complexes, such as phthalocyanine, methylene blue, Prussian blue, ferrocene, and the like.

Two pieces of information turned out to be valuable for the successive investigation: first, optical microscopy on thin sections of the flints clearly showed that the areas of the specimen pervaded by the blue color are confined to the surface of the object (Fig. 2), pointing to an external contamination penetrating the sample, and that the optically stimulated areas emitting the blue coloration are spatially related to the concentrations of Fe-hydroxides under the surface (Fig. 2) and with the silica matrix (Fig. 3). The blue-emitting areas also show a weak blue luminescence if irradiated by UV light, whereas no effect is observed when the sample is excited by cathodoluminescence.

Furthermore, gas chromatography performed on the molecular species thermally desorbed from the flint surface constantly showed a pervasive contamination by aliphatic hydrocarbons, with a molecular weight distribution similar to that of oil-derived compounds. Together with the aliphatic molecules, several other hydrocarbons were detected, mainly phthalates and butylated hydroxytoluene (BHT), possibly released by the plastic bags and pads surrounding the samples. Traces of the same molecular species were detected on the surface of bones and ceramics stored in the deposit cabinets together with the lithic artifacts.

Since the preliminary analytical results allowed no detection of pigmenting ions or molecules, it was decided to perform a complete extraction and concentration of the escaping blue compounds, using acetone at 60 °C (Fig. 1b). The concentrated dark blue solution we obtained after the solvent evaporation was first analyzed by ^{1}H and ^{13}C NMR and by FT-IR. Unfortunately, only the phthalate signals were clearly observed, pointing out the extremely low concentration of the pigmenting molecules. Subsequent analysis of the concentrated solution by liquid chromatography with both a diode array UV–Vis detector and a high resolution time-of-flight mass spectrometric detector (reverse phase HPLC/DAD/ESI-Q-TOF-MS) showed the presence of a group of "blue molecules" similarly retained in the chromatographic column: two main peaks (t_R 10.82 and 11.13) together with one minor peak (t_R 11.47) and other minor signals in the noise.

The DAD spectra of the three well detectable peaks evidenced a characteristic sharp absorption band of all pigmenting molecules (λ_{max} 622, 608, and 595 nm, respectively) with a shoulder at lower wavelength. This behavior is typical of the poly-aminophenylmethane dyes [12].

ESI-Q-TOF/MS analysis showed (a) significant small differences between the exact molecular masses of these molecules and (b) an identical distribution of the isotopic ion signals. The m/z values of the monoisotopic M$^+$ ions of the three pigmenting molecules were 528.3390, 530.3541, and 532.3701 amu, respectively, possibly related to very similar molecules with different degrees of unsaturation. Quantitative modeling of the observed isotopic distribution (M$^+$ peaks) based on natural carbon isotopes occurrences was only possible by assuming molecules with elemental composition $C_{37}H_{42}N_3^+$ (exact mass 528.3378 amu), $C_{37}H_{44}N_3^+$ (exact mass 530.3535 amu), and $C_{37}H_{46}N_3^+$ (exact mass 532.3692 amu), respectively.

As a consequence, the compatible molecular structures reported in Fig. 4 are proposed, assuming the usual cationic configuration of most triphenylmethane dyes. The structures correspond to the novel compounds 6-(bis(2,2,4-trimethyl-1,2-dihydroquinolin-6-yl)methylene)-2,2,4-trimethyl-2,6-dihydroquinolinium (proposed name Juliet Blue, in honor of Verona and Shakespeare, Fig. 4a), 2,2,4-trimethyl-6-((2,2,4-trimethyl-1,2,3,4-tetrahydroquinolin-6-yl)(2,2,4-trimethyl-1,2-dihydroquinolin-6-yl)methylene)-2,6-dihydroquinolinium (Romeo Blue, Fig. 4b), and

Fig. 2 Optical microscopy photographs: **a** transmitted plane-polarized light and **b** reflected light in slightly displaced crossed Nichols mode. Both surfaces of the flint shows a *light blue coloration* related to the penetration of the *blue color* at a depth of about 20–120 μm. The distribution of the *blue color* visible in Fig. 2b is closely related to the distribution of the Fe-hydroxides layer below the surface (Fig. 2a)
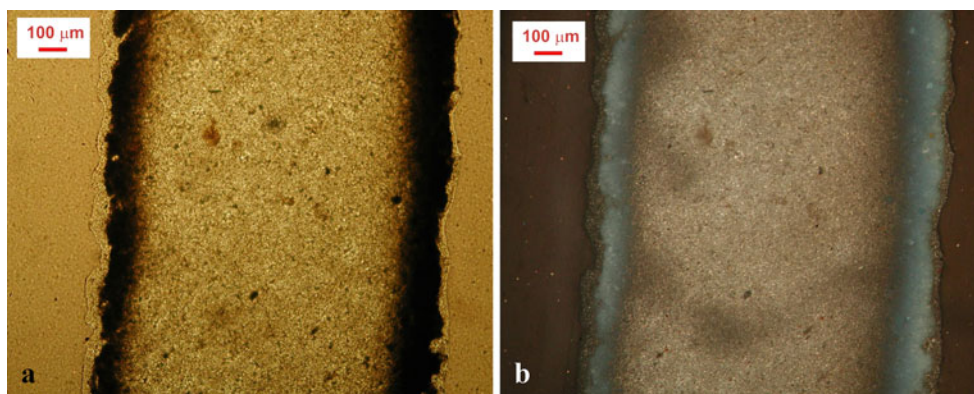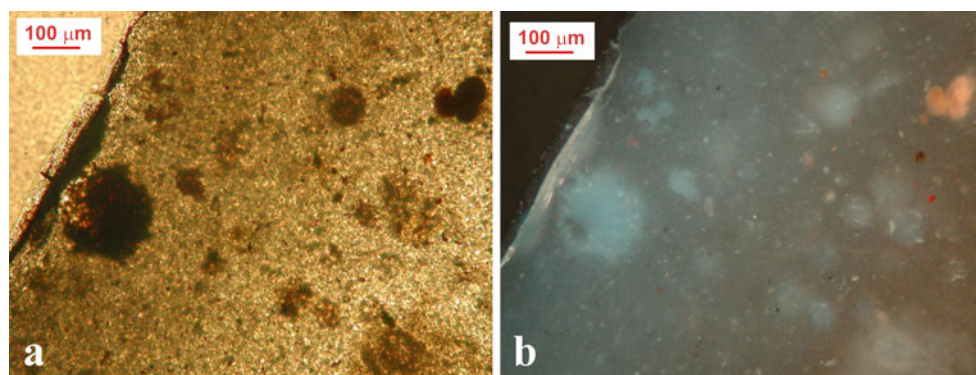
Fig. 3 Optical microscopy photographs: **a** transmitted plane-polarized light and **b** reflected light in slightly displaced crossed Nichols. Careful comparison between the two pictures clearly shows that the *blue coloration* is stronger in the areas with inclusions rich in Fe-hydroxides

6-(bis(2,2,4-trimethyl-1,2,3,4-tetrahydroquinolin-6-yl)methylene)-2,2,4-trimethyl-2,6-dihydroquinolinium (Flint Blue, Fig. 4c).

The color vs pH dependence of the extracted solution agrees with the hypothesis that it contains some triaminophenylmethane-like molecules. By addition of HCl, the blue solution becomes green (pH=1–1.5) and then light yellow (pH<0.5). This behavior, completely reversible, can be related to the protonation of the two neutral nitrogen atoms in the molecule. At higher pH values (pH=11–12, by NaOH), the blue solution becomes colorless/pale yellow to indicate the expected hydrolytic formation of the carbinol (a triphenylmethanol, with H^+ elimination) and the consequent loss of the electronic delocalization. These three acid-base equilibria are well described for the pararosaniline derivative dyes (for

instance, the pKa values of Coomassie Blue G are 1.15, 1.8, and 12.4 [13]).

The proposed structures were subsequently confirmed by an accurate MS/MS fragmentation analysis of the molecular ions M^+ (see the "Materials and methods" section), which allowed the complete assignment of all ion fragments. At lower collision voltage (up to 50 V), M^+ fragmentation is limited to the progressive loss of the methyl substituents (CH_4, C_2H_6 or $\cdot CH_3$) to form the corresponding triphenylmethane ions with mono- or dimethylquinoline branches (aromatic structures, stabilized by π resonance): m/z 513.31 ($-\cdot CH_3$) and 498.29 ($-C_2H_6$) for Juliet Blue; m/z 515.33 ($-\cdot CH_3$) and 500.31 ($-C_2H_6$) for Romeo Blue; and m/z 517.35 ($-\cdot CH_3$) and 502.32 ($-C_2H_6$) for Flint Blue. At higher fragmentation energy (collision voltage 70 V), the further progressive demethylation of the three pigmenting molecules can be observed by the signals at m/z 482.26, 468.24, and 453.22 (Juliet Blue); 499.30, 485.28, and 470.25 (Romeo Blue); and 516.34, 502.32, and 586.29 (Flint Blue). Under these conditions, only minor evidences of the cleavage of the molecular branches appear, whereas they are slightly more detectable at higher collision voltage (100 V): m/z at 323.15, 309.14, 297.14, and 285.14. It is worth to notice that these masses correspond exactly to the cleavage of the demethylated branch of the proposed structures and no signals can be assigned to fragments formed by the direct loss of 2,2,4-trimethyl-1,2-dihydroquinoline (TMQ, exact mass 173.12 amu, Fig. 4d). The sole presence of the dimethylquinoline signals (m/z 157.09 and 158.10 for M^{+} and MH^+, respectively) observed at lower m/z range of MS/MS spectra of all these molecules, further supports a fragmentation pattern, via demethylated species formation [14, 15], compatible with the proposed structures.

Further confirmation of the structural arrangement of the blue compounds is derived from the direct analysis of the molecules desorbed by the black rubber pads lining the floor of the drawers of the cabinets where the archaeological collection is stored. GC-MS analysis of both the thermally and the hexane-desorbed molecules show the presence of aliphatic hydrocarbons, phthalates, and of other
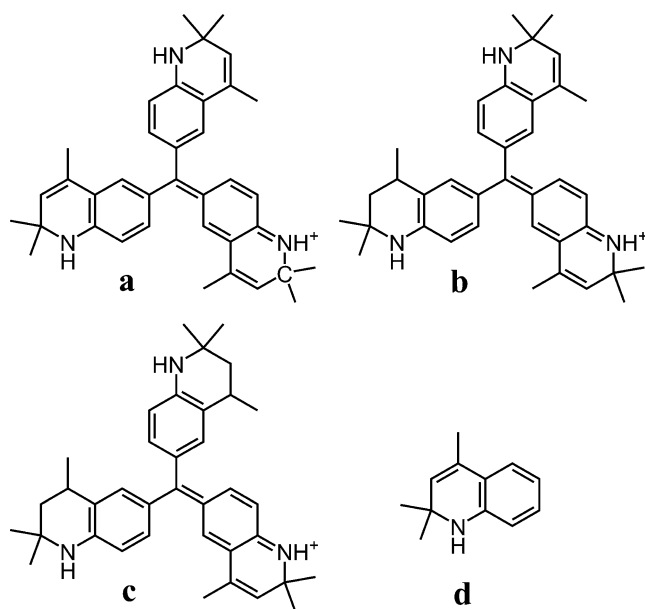

Fig. 4 Structures of **a–c** the novel blue pigmenting molecules and **d** the hypothesized precursor 2,2,4-trimethyl-1,2-dihydroquinoline (TMQ). Proposed names: **a** Juliet Blue; **b** Romeo Blue; and **c** Flint Blue

common additives used as antioxidant in polymeric materials [16, 17] such as TMQ and BHT. TMQ, whose fragmentation ions (*m/z* 157.09 and 158.10 amu) were also observed during LC-MS/MS analysis of all pigmenting molecules, represents the building block of the novel blue dyes possibly assembled within the pores of the silica. Plausible mechanisms for the formation of a tris derivative of TMQ may involve any carbonyl additions after the interaction of the dihydroquinoline, via hydrogen bond, with the SiO_2 surface or with the Fe-hydroxides inclusions. The latter interaction seems to be more likely based on the spatial relationship between the dye and the Fe-hydroxides observed by optical microscopy (Figs. 2a and 3a). Indeed, the catalytic action of the Fe cations in the formation of the molecules is the most likely mechanism, although the possibility of direct or indirect bacterial enhancement of the catalysis within the pores cannot be completely ruled out. In fact several bacterial species were extracted and identified from the flints' surface (mainly *Stenotrophomonas* sp. and *Sphingobacterium multivorum*), although none of these species is reported to produce blue pigmenting molecules. Tentative reproduction of the discoloration process in the laboratory under controlled conditions is in progress.

The blue molecules are somehow structurally related to the threefold symmetry compounds known as triphenylmethane dyes [18], which include well-known pararosaniline derivatives such as Fuchsine, Crystal Violet, Methyl Violet, Aniline Blue, and Victoria Blue. However, a triphenylmethane compound with quinoline-like constituents has never been synthesized. The flint-catalyzed molecules have evidently a strong pigmenting power allowing their visual detection even at extremely low concentration. Furthermore, they have strong affinity with the silica surface and high stability both in solution and after absorption on the silica pore surface.

Conclusions

Besides leading to the discovery of the new dyes, the investigation represents a clear example of the unpredictable effects encountered in the conservation of the cultural heritage [4]. Were it not for the color change of some of the flints acting as an unpredictable sensor, the weak contamination of the archaeological objects would probably have escaped detection in absence of thorough scientific analyses. The adsorbed organic compounds could even bias analytical results, especially those obtained with techniques

probing extremely low amount of material. Exceptional care is thus recommended when planning and organizing both the preservation and the analysis of cultural heritage materials. The management of collections made of complex man-produced materials, both ancient and industrial, summed to the large variety of degradation processes acting upon them demands that the huge technical and methodological potential of modern materials science must be exploited at the best to define conservation strategies.

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