Hybrid Pigments from Anthocyanin Analogue and Synthetic Clay Minerals

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ABSTRACT: Flavylium cations are synthetic analogues of anthocyanins, the natural plant pigments that are responsible for the majority of the red, blue, and purple colors of flowers, fruits, and leaves. Unlike anthocyanins, the properties and reactivity of flavylium cations can be manipulated by the nature and position of substituents on the flavylium cation chromophore. Currently, the most promising strategies for stabilizing the color of anthocyanins and flavylium cations appear to be to intercalate and/or adsorb them on solid surfaces and/or in confined spaces. We report here that hybrid pigments with improved thermal stability, fluorescence, and attractive colors are produced by the cation-exchange-mediated adsorption of flavylium cations (FL) on two synthetic clays, the mica-montmorillonite SYn-1, and the laponite SYnL-1. Compared to the FL/SYn-1 hybrid pigments, the FL/SYnL-1 pigments exhibited improved thermal stability as judged by color retention, better preferential adsorption of the cationic form of FL1 at neutral to mildly basic pH (pH 7–8), and lower susceptibility to color changes at pH 10. Although both clays adsorb the cationic form on their external surfaces, SYnL-1 gave more evidence of adsorption in the interlayer regions of the clay. This interlayer adsorption appears to be the contributing factor to the better properties of the FL/SYnL-1 hybrid pigments, pointing to this clay to be a promising inorganic matrix for the development of brightly colored, thermally more stable hybrid pigments based on cationic analogues of natural plant pigments.

1. INTRODUCTION

Flavylium cations are synthetic analogues of anthocyanins, the natural plant pigments responsible for the majority of the red, blue, and purple colors of flowers, fruits, and leaves. Flavylium cations are excellent alternatives for anthocyanins since their chemical and photochemical reactivity often closely mimics those of the natural anthocyanins. Moreover, they have the advantage of versatility since the introduction of substituents on the flavylium chromophore can be used to manipulate their stability and reactivity. Due to their antioxidant properties and color, both anthocyanins and flavylium cations have great potential for practical applications in fields such as pharmaceuticals, foods and beverages, and cosmetics. However, these applications are hampered by the limited persistence of their color, which can be affected by pH, light, oxygen, temperature, among others. For example, in foodstuffs, the application of anthocyanins as colorants is typically restricted to foods or beverages with pH ≤3, where the dominant species present is the highly colored flavylium cation. At a higher pH, nucleophilic attack of water on the colored flavylium cation chromophore forms a near-colorless hemiketal form, which can subsequently undergo ring-opening tautomerism to give near-colorless hydroxychalcones.

A rather obvious strategy for stabilizing the color of natural anthocyanins or flavylium cations is to isolate them in an environment in which they are no longer susceptible to attack by water. Particularly interesting for this purpose are inorganic matrices such as clays, mesoporous materials, and zeolites, in which organic molecules can intercalate in confined spaces or adsorb on surfaces. A number of recent studies have focused on the adsorption of anthocyanins or flavylium cations on clay minerals to obtain dye/clay hybrid pigments with improved color and stability. These include reports of highly fluorescent hybrid pigments derived from flavylium cations and fibrous clays with enhanced chemical and thermal stability and stable hybrid pigments prepared from anthocyanins and several clay minerals that undergo reversible pH-dependent color changes.

In this work, we report the properties of hybrid pigments prepared from a series of seven substituted flavylium cations...
and two different lamellar clay minerals: a synthetic laponite (Laponite RD, SYnL-1) and a synthetic mica-montmorillonite (Barasym SSM-100, SYn-1). Both of the synthetic clays have excellent surface properties but exhibit differences with regard to swelling and the distribution of charges on their surfaces that can potentially contribute to differences in the adsorption of the flavylum cations and the stability of their color to external influences such as pH and temperature.

Laponite is a synthetic hectorite-type clay mineral consisting of two tetrahedral silicate layers connected to an octahedral Mg2+ layer via oxygen atoms.20–23 Its highly expansible structure is described as strongly anisotropic platelets or disks that can be exfoliated in an aqueous medium in low concentrations.20–23 The average size of the individual laponite particles is approximately 25 nm in diameter and 0.92 nm in thickness.20,22,23 The net negative charge on the faces of these disks is due to isomorphous substitutions of Mg2+ by Li+ in the octahedral layer and is compensated by exchangeable hydrated cations such as Na+ in the interlayer space between the faces.20–22 On the edges of the platelets, the charge can vary due to the presence of amphoteric SiOH and/or MgOH sites with pH-dependent charges.20–22 SYn-1, on the other hand, is a synthetic 2:1 phyllosilicate that possesses random layering of expandable and nonexpandable dioctahedral layers in a 1–2 ratio.24–26 The layers of SYn-1 present negative charges due to partial isomorphous substitutions of higher valence cations with lower valence cations of similar size, as for example replacement of Si4+ by Al3+ in the tetrahedral sheets.27 The external surface sites and those in the expandable layers participate in cation exchange and can therefore host cationic molecules.24,26 Despite the presence of expandable layers, compared to laponite, SYn-1 is essentially a nonswelling clay.23,25–27 The adsorption of dyes on SYn-1 has been shown to produce promising hybrid materials with interesting luminescence and aggregation properties.30–32 Hybrid materials prepared from organic dyes and laponite have attractive colors and stability reminiscent of those of the ancient Maya Blue pigment.25,53

2. RESULTS AND DISCUSSION

2.1. Preparation of the FL/Clay Samples. The seven FL used in this work have substituents of similar nature in a variety of positions on the flavylum chromophore to examine whether there is an influence of FL substitution pattern on the adsorption process and/or on the properties of the FL/clay hybrids such as color and color stability. During the adsorption process, the total amounts of FL added to the clays (ca. 10 mmol/100 g) were well below the cation-exchange capacities (CEC) of these clays, which have been reported to be 140 meq/100 g for SYn-132 and 60 meq/100 g for SYnL-125,34. In addition, because all of the FL employed are soluble in water, exhaustive washing with acidic water should remove any excess or weakly physisorbed FL, leaving only the more strongly bound FL. Indeed, the final amounts of FL still adsorbed after the washing step (ca. 90–100%) were very similar for all FL and for both clays, showing that the substituents had little or no effect on the adsorption. This in itself suggests that ion exchange is potentially the most important mode of interaction of these cationic dyes with these synthetic clays. Because of the rather efficient adsorption of the flavylum ions on the clays, all of the FL/clay samples presented intense and attractive colors.

2.2. Characterization Studies. The powder X-ray diffractograms (XRDs) of the samples showed little or no differences from those of the raw clays (Figures 1, S1 and S2 of the Supporting Information). Table 1 shows the basal spacing

(\(d_{001}\)) values, calculated for the clays and FL/clay samples. Although the acidic medium employed in the adsorption process did not change the basal spacing of the clays, there was a decrease in the intensity of the \(d_{001}\) peak (more pronounced for the FL/SYn-1 samples), attributed to the delamination of the clay layers.32 The observed reflection peaks of SYn-1 and SYnL-1 correspond to the basal spacings of 11.3 and 13.5 Å, respectively. The basal spacing values of the FL/SYn-1 samples were similar to that of SYn-1 (Table 1 and Figure S2a), implying that the FL were adsorbed predominantly on the external surfaces of SYn-1. This is in agreement with the results reported for isocyanine dye/clay hybrids, for which the basal spacing remained the same as that of the raw clay (SYn-1 or laponite) with up to 10% isocyanine dye/clay.22,32 Indeed, as shown previously in tetracycline adsorption studies,18,24 SYn-1 is essentially a nonswelling clay. In contrast, laponite clays do present good expansion properties upon intercalating tetracycline35 or anthocyanins.56 In accordance with this, our FL/SYNL-1 samples showed a slight increase in the \(d_{001}\) values relative to the raw clay (Table 1 and Figure S2b), pointing to some intercalation between the layers of SYnL-1 in addition to adsorption on the external surface. Subtracting the 9.2 Å silicate layer thickness of laponite22,23 from the range of basal spacings found for the FL/SYnL-1 samples (13.9–15.0 Å; Table 1) gives estimates of 4.7–5.8 Å for the interlayer expansion. This value is quite compatible with a thickness of ca. 3.7 Å estimated for FL (without considering additional contributions from partial hydration or nonplanarity of the FL chromophore) adsorbed on palygorskite14 or sepiolite.15

Adsorption of FL primarily on the external surfaces of SYn-1, but in a significant amount on both the external surfaces and in the interlayer region of SYnL-1, as illustrated in Figure 2, would be consistent with these structural results, again pointing to a cation-exchange mechanism for the adsorption of the FL.

Significant interlayer adsorption of the dye in the case of SYnL-1 should lead to morphological changes in the clay particles not attributable to treatment with HCl. SEM micrographs of FL/SYnL-1 and SYnL-1 treated with the same acidic medium employed for the adsorption of the FL are shown in Figure 3. SYnL-1 itself exhibited microagglomerates, with flat surfaces and irregular shapes and sizes, consistent with the report37 that laponite agglomerates via the stacking of elementary layers form large clusters. In contrast, the micrographs of FL/SYnL-1 show a large modification of the particle shapes and the external surface relative to SYnL-1 resulting from the FL adsorption on the clay.
Infrared spectra of the SYn-1 and SYnL-1 clays, the four similarly substituted dyes FL2, FL5, FL6, and FL7, and the corresponding FL/clay samples are presented in Figure 4. The raw SYn-1 clay exhibits absorption bands at 535, 620, 757, 820, 930 (shoulder), 1030 (most intense band), 1405 (shoulder), 1438, 1639, 3447, and 3629 cm$^{-1}$. Raw SYnL-1 clay exhibits bands at 654, 1011 (most intense band), 1639, 3454, and 3627 cm$^{-1}$. For SYn-1, the band at 1438 cm$^{-1}$ and the shoulder at 1405 cm$^{-1}$ correspond to vibrations of the exchangeable NH$_4^+$ cation in its interlayer. The shoulder at 930 cm$^{-1}$ corresponds to Al–Al–OH, and the bands at 620 cm$^{-1}$ (characteristic of muscovite) and 757 and 820 cm$^{-1}$ (characteristic of montmorillonite) correspond to Al–O out-of-plane and Al–O–Si in-plane bending vibrations; the band at 535 cm$^{-1}$ is also associated with Al–O–Si bending. The shoulder at 930 cm$^{-1}$ corresponds to Al–Al–OH, and the bands at 620 cm$^{-1}$ (characteristic of muscovite) and 757 and 820 cm$^{-1}$ (characteristic of montmorillonite) correspond to Al–O out-of-plane and Al–O–Si in-plane bending vibrations; the band at 535 cm$^{-1}$ is also associated with Al–O–Si bending. For SYnL-1, the band at 654 cm$^{-1}$ corresponds to the Mg–OH bending vibrations. The most intense bands correspond to the Si–O stretching vibration and are located at 1030 cm$^{-1}$ (SYn-1) and 1011 cm$^{-1}$ (SYnL-1). The bands in the range 3700–3000 cm$^{-1}$, along with a band at 1639 cm$^{-1}$, are the respective OH-stretching (including all OH groups coordinated to different octahedral cations) and bending vibrations of water molecules present in the two clays.

Table 1. Basal Spacing, $d_{(001)}$ (Å), of the Clays and FL/Clay Samples

| Clay   | FL1 | FL2 | FL3 | FL4 | FL5 | FL6 | FL7 |
|--------|-----|-----|-----|-----|-----|-----|-----|
| SYn-1  | 11.3| 11.5| 11.6| 11.6| 11.6| 11.4| 11.5|
| SYnL-1 | 13.5| 15.0| 14.6| 13.9| 14.5| 14.9| 13.9|

As shown in Figure 4c–e, additional IR bands clearly attributable to the FL cations adsorbed on the FL/clay samples were detected only in the range of 1750–1130 cm$^{-1}$, a spectral region that includes a variety of C–O, C–C, and C–H vibrations. The intensities of the FL bands were quite weak in the FL/clay samples, reflecting the relatively small amount of FL adsorbed in relation to clay, and spectral shifts compared to free FL were small. In the literature, the IR bands of flavlylium cations at 1182 and 1302 cm$^{-1}$ have been attributed to the C–H bending vibrations, while the bands at 1231 and 1272 cm$^{-1}$ may correspond to the CO skeletal stretching vibrations and the 1344–1354 cm$^{-1}$ region and the bands at 1506 and 1529 cm$^{-1}$ possibly to the C–C stretching. Although bands close to 1378 and at 1577 cm$^{-1}$ were tentatively attributed to the C–C stretching, a band at 1377 cm$^{-1}$ has also been attributed to an O–H in-plane deformation in polyphenols. However, the fact that these bands are only observed in our case for FL5 and FL6 and the respective FL/clay samples (which present a small shift to 1383–1384 and
1583 cm⁻¹) suggests an association of these vibrations with a 7-methoxy-substituted A ring of the flavylium cation.

Steady-state fluorescence excitation and emission spectra of the FL/clay samples are shown in Figure 5. Because FL1 and FL4 are essentially nonfluorescent in solution, the observation of fluorescence from FL1 adsorbed on SYn-1 and SYnL-1 is particularly noteworthy, clearly indicating a significant increase in the fluorescence quantum yields of these two dyes upon interaction with the clays. Although the methyl group in the 4-position of FL3 and FL4 blocks loss of color due to hydration, deprotonation of the hydroxyl group(s) occurs above pH 4. Therefore, all of the FL used in this work change color at basic pH.

2.3. Color Resistance to Temperature. The FL are typically thermally unstable at high temperatures. For example, FL1 undergoes substantial degradation at 120 °C in <2 h. In contrast, the FL/clay samples, FL1/SYnL-1 and FL1/SYn-1, retained their characteristic colors for at least 28 h at 100 °C and for another 20 h upon raising the temperature to 125 °C (Figure 6). On the other hand, some degradation was observed at the end of this thermal treatment for FL1/SYn-1 and, more significantly, for FL7/SYn-1 (Figure 6).

2.4. Effect of a pH Change on Color. In an acidic medium (pH < 3), the cation is the dominant form of the FL. In aqueous solution, FL1 and FL5 begin to lose their color above about pH 3 due to nucleophilic attack by water, leading to the formation of the corresponding hemiketal and subsequent ring-opening tautomerism to the hydroxychalcone form. Although the methyl group in the 4-position of FL3 and FL4 blocks loss of color due to hydration, deprotonation of the hydroxyl group(s) occurs above pH 4. On the other hand, FL2, FL6, and FL7 are prone to color changes due to both nucleophilic attack by water and deprotonation, as well as deprotonation of the hydroxychalcones formed from the hemiketal. Therefore, all of the FL used in this work change color at basic pH.

Figure 7 shows the impact of a 24 h immersion of the FL/clay samples in pH 10 aqueous buffer on their color after drying in vacuum. Ultraviolet—visible (UV—vis) absorption spectra of aqueous solutions of the FL in the same pH range and remission spectra of the FL/clay samples are provided in Figures S3 and S4, respectively, of the Supporting Information. The corresponding CIE 2000 color coordinates are listed in Tables S1, S2, S3, and S4 of the Supporting Information.
diff erences in Table S4 of the Supporting Information. No leaching of the FL from the clay into the pH 10 aqueous medium was observed for any of the samples. The colors of the FL/SYN-1 samples prepared from the FL that possess hydroxyl groups changed completely, indicating that the adsorbed FL continued to undergo deprotonation (Figure 7a and Table S2 of the Supporting Information). Although a new band corresponding to the hydrated forms of FL1 and FL5 appeared around 380 nm (Figure S2 of the Supporting Information), a significant fraction of the cationic form of the FL was still present for the FL1/SYN-1 and FL5/SYN-1 samples (Figures 7a and S2 of the Supporting Information), indicating that these two adsorbed FL are less prone to hydration. In contrast, the colors of most of the FL/SYNL-1 samples faded slightly or became slightly more intense, maintaining substantial color (Figure 7b) despite minor changes in brightness, saturation, and hue (Table S3 of the Supporting Information). Therefore, the FL adsorbed on SYNL-1 were less prone to hydration and deprotonation. The two exceptions that did show much greater changes in color, i.e., FL6/SYNL-1 and FL7/SYNL-1, have a 4′-OH group in the B-ring of the FL, suggesting that this group can still be deprotonated.

2.5. Preferential Adsorption of the Cationic Form of the FL. The stabilization of the cationic form of FL1 upon adsorption on the synthetic clays was demonstrated by experiments in which FL1 was preincubated in pH 7 or pH 8 phosphate buffer to convert it to the equilibrium mixture of nearly colorless hydrated species prior to the addition of the SYN-1 or SYNL-1 clays. Following the addition of SYN-1 (Figure 8a) or SYNL-1 (Figure 8b), however, the suspended clay exhibited a visible color change within 10 min and gradually acquired the coloration of the adsorbed FL1 cation over a period of about a day, followed by stabilization of the color during the remainder of the experiment (27 days total).

At pH 7 and pH 8 in the buffer, the flavylum cationic form of FL1 is completely converted to the hydrated forms, with the slightly more yellow hue of the solution at pH 8, suggesting partial deprotonation of the hydroxychalcone form at pH 8 relative to pH 7. Indeed, upon the addition of the clays, the rate of appearance of the coloration at pH 8 was slightly slower than that at pH 7. However, the FL1/clay colors at long times (27 days) were similar at the two pH values and much more intense in the case of SYNL-1 clay. The results suggest that under these conditions the hydrated forms are initially adsorbed on the clay and then subsequently converted to the cationic form due to interactions with the acidic sites of the SYN-1 and SYNL-1 clays. Laponite clays have pH-independent negative surface charges on their faces and pH-dependent positive surface charges on their edges, with a point of zero charge (for the edges) at pH 10.21,47,48 Mica-montmorillonite clay, like SYN-1, does not present a point of zero charge.29 Isomorphic substitution of Al3+ for Si4+ in the tetrahedral layers generates negative charges that are balanced by interlayer cations, resulting in the generation of acid sites.27,49 Acid sites can also occur at edge defects located in the intralayer.49 At neutral pH, 7% of the cation-exchange capacity of SYN-1 was reported to consist of acid sites capable of protonating methylene blue.24

3. CONCLUSIONS

Hybrid pigments with improved thermal stability, fluorescence, and attractive colors were produced by the cation-exchange-mediated adsorption of flavylum cations (FL) on synthetic mica-montmorillonite clay (SYN-1) and synthetic laponite clay (SYNL-1). Compared to the FL/SYN-1 composites, the FL/SYNL-1 composites exhibited improved thermal stability of the FL, better preferential adsorption of the cationic form of FL1 at neutral to mildly basic pH (pH 7–8), and lower susceptibility to color changes at pH 10. Although both clays adsorb the cationic form on their external surfaces, SYNL-1 gave more evidence of adsorption in the interlayer regions of the clay. Interlayer adsorption appears to be a contributing
The chloride salts of the flavylium cations (FL) used in this work (Scheme 1) were available from previous studies of the group.\textsuperscript{35,50,51} The synthetic mica-montmorillonite (Barasym SSM-100) (SYn-1) from the Source Clays Repository of the Clay Minerals Society was generously provided by Dr. Miguel Neumann, IQSC-USP, São Carlos, Brazil, and was used as received. The chemical composition, characterization, and properties of this clay have been reported.\textsuperscript{32,52–54} Laponite RD (SYnL-1), Southern Clay Products Inc., was purchased from the Source Clays Repository of the Clay Minerals Society and used as received. Its composition, characterization, and properties also have been described.\textsuperscript{55,56} Hydrochloric acid (HCl, Vetec) was used as received. Ultrapure water was used for the preparation of all aqueous solutions.

4.2. Preparation of the FL/Clay Samples. Aliquots of solutions of the flavylium cations (FL) in water containing 0.010 mol dm\textsuperscript{-3} HCl (pH 2, to avoid hydration of the FL or deprotonation of OH groups when present) were added to tubes containing a weighed amount of clay powder. The initial FL/clay ratios utilized were ca. 0.1 mmol g\textsuperscript{-1}. The resulting suspensions were stirred for 24 h in the dark at room temperature, centrifuged, solid-washed exhaustively with HCl-aqueous solutions.

4.3. X-ray Diffraction. Powder X-ray diffractograms of the clays (SYn-1 and SYnL-1) and the FL/clay samples were determined with a Bruker D2 phase diffractometer using Cu Kα radiation (1.5418 Å, 30 kV, 15 mA) employing a scan step of 0.05°. Basal spacings for the clays and FL/clay samples were calculated from the diffraction angle using Bragg’s equation: \[ \lambda = 2d \sin \theta \] where \( \lambda \) is the wavelength of Cu Kα radiation (1.5418 Å), \( d \) is the basal spacing, and \( \theta \) is the diffraction angle.

4.4. Scanning Electron Microscopy. Scanning electron microscopy (SEM) images were obtained with a JEOL JSM 7401F (FEG) equipment with a small clay sample spread directly on a carbon film support. The clay sample without adsorbed dye was treated with HCl in the same manner as the dye/clay samples in Section 4.2 to eliminate the possibility that changes might be due to HCl rather than the adsorption of the FL.

4.5. Spectroscopic Measurements. Infrared measurements were performed by adding about 1.5 mg of solid sample to approximately 150 mg of dry KBr in a small agate mortar and mixing by grinding. The resulting powder was pressed into a pellet using a hydraulic press (Caver, model 3912, Wabash). Infrared spectra of the pellets were collected using a Bruker Vector 22 FTIR spectrophotometer in the frequency range of 4000–500 cm\textsuperscript{-1} and 32 scans at 0.2–0.3 cm\textsuperscript{-1} digital resolution.

The UV–vis-diffuse reflectance (DR) spectra were measured with a Varian Cary 50 UV–vis Bio spectrophotometer equipped with a Barretino diffuse reflectance probe (Harrick Scientific Products, Inc.). Due to the large amount of FL adsorbed, the samples were diluted in barium sulfate. The remission spectra were obtained from the UV–vis-DR by employing the Kubelka–Munk equation:

\[ F(R) = \frac{(1 - R)^2}{2R} \] where \( R \) is the absolute reflectance at each wavelength.

CIELAB color coordinates (CIE \( L^*a^*b^* \))\textsuperscript{55} were obtained from the UV–vis-DR measurements using the software Agilent Cary WinUV Color. Natural daylight illuminant CIE D65 was set as a standard illuminant and the observer angle was 2°. For CIELAB color measurements, the samples were not diluted in barium sulfate. CIE \( L^*C^*h^* \) color coordinates were obtained from the CIE \( L^*a^*b^* \) data by employing the following relationships:

\[ \begin{align*}
L^* &= L^* \\
C^* &= (a^*^2 + b^*^2)^{0.5} \\
h^* &= \arctan(b^*/a^*)
\end{align*} \]

where these represent the color attributes brightness (lightness, \( L^* \)), saturation (chroma, \( C^* \)), and hue (\( h^* \)).

Steady-state fluorescence measurements were performed with a Hitachi F-4500 fluorescence spectrophotometer, which was equipped with a solid sample holder for measurements on the powdered clay samples. The excitation and emission wavelengths are indicated in the figure legends. Excitation and emission bandwidths were either 2.5 or 5.0 nm depending on the sample fluorescence intensity.

4.6. pH Sensitivity and Thermal Stability Tests. The effect of pH on the color of the FL/clay samples was investigated by the addition of ca. 15 mg of the FL/clay
samples to 5 mL of 10 mmol dm\(^{-3}\) sodium borate buffer solution, pH 10. The samples were stirred for 24 h and then centrifuged to separate the solid samples, which were dried under vacuum. The UV-vis-DR spectra as well as color coordinates were obtained to verify any spectral or color variations. To investigate the selectivity of the adsorption of the cationic form of FL1 to the clays, FL1 was added to 10 mmol dm\(^{-3}\) sodium phosphate buffer solutions, pH 7 and 8, and allowed to hydrate, indicated by the substantial loss of the color of the solution, followed by the addition of 0.05 g of each clay. The samples were maintained under constant stirring at room temperature (ca. 20 °C) and digital images were recorded as a function of the time to follow the color changes.

Thermal stability was investigated by heating FL1/clay and FL7/clay samples under vacuum at 100 °C for 28 h, followed by heating for an additional 20 h at 125 °C. Digital images were used to verify any color changes.

**ASSOCIATED CONTENT**

1. **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c03354.

Powder X-ray diffractograms of the clays and all of the FL/clay hybrid pigments. pH-dependent UV-vis absorption spectra of the FL in aqueous solution. UV-vis-DR spectra (Kubelka-Munk mode), CIE \(L^*a*b*\) and \(C^*h^*\) coordinate data and total color differences for the FL/SYN-L-1 and FL/SYN-1 samples before and after heat treatment or immersion in a pH 10 aqueous buffer medium, and total color differences during the selective adsorption experiments (PDF).

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**Notes**

The authors declare no competing financial interest.

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