Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 252 (2021) 119455

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

# Complexes of carboxylato pillar[6]arene with Brooker-type merocyanines: Spectral properties, p$K_a$ shifts and the design of a displacement assay for trimethyl lysine

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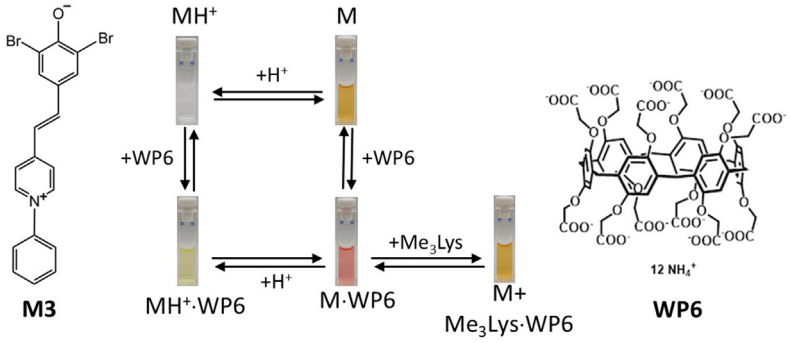
## HIGHLIGHTS

- The complexation of three pyridinium phenolate betain dyes with the water soluble pillararene WP6 was studied.
- Both the phenol and the phenolate forms of the dyes form stable complexes with WP6.
- The pKa values of the phenol forms of the dyes increase by 1.1–1.6 units on complexation.
- One of the dye-WP6 complexes functioned as an indicator displacement assay for Me3Lys.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 6 October 2020
Received in revised form 5 January 2021
Accepted 5 January 2021
Available online 8 January 2021

*Keywords:*
Pyridinium phenolate dye
Water-soluble pillararene
Solvatochromism
Host–guest complex
Acidic dissociation constant
Hydrophobic effect
Indicator displacement assay

## ABSTRACT

The supramolecular complexes of three strongly solvatochromic dyes, Brooker's merocyanine (M1) and its two derivatives (M2, M3) with carboxylato pillar[6]arene (WP6) were studied in aqueous solutions. The dye-WP6 mixtures were described in terms of four equilibrium reactions: the acidic dissociations of the pyridinium phenols into the zwitterionic phenolates, the acidic dissociations of the complexed phenols, the bindings of the phenol form dyes to WP6 and the bindings of the phenolates to WP6. The equilibrium constants were determined by an analysis of the absorption spectra. It was found that the acidity of the phenol form merocyanines were largely reduced on complexation, p$K_a$ shifts of 1.1–1.6 units were observed. In neutral solutions, the complexes of the phenol forms of M1 and M2 were dominant, in contrast to the more acidic M3 (a dibromo derivative), of which the phenolate complex was more stable. Comparing the spectral properties, the binding constants and the p$K_a$-s of the dye-WP6 complexes, the complex M3·WP6 was chosen to be tested as a displacement assay. It was demonstrated that this complex functioned as a colorimetric indicator displacement assay which discriminated trimethyl lysine from other lysine derivatives.



## 1. Introduction

Brooker's merocyanine (M1 in Fig. 1) is a pyridinium phenolate dye which exhibits an extremely strong solvatochromic behavior

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https://doi.org/10.1016/j.saa.2021.119455
1386-1425/

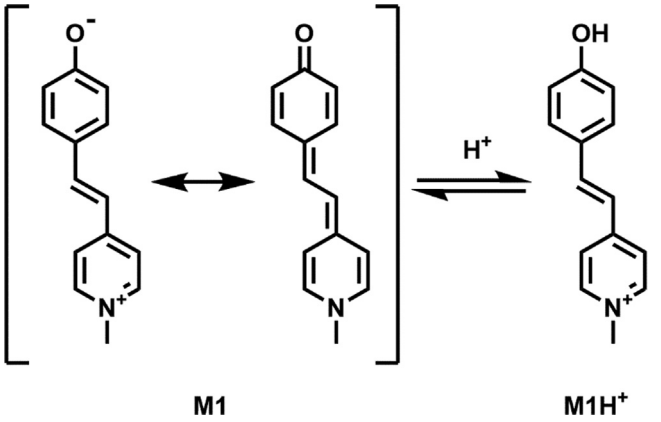


**Fig. 1.** The phenolate and phenol forms of Brooker's merocyanine. The phenolate is a hybride of a zwitterionic and a neutral mesomeric structure.

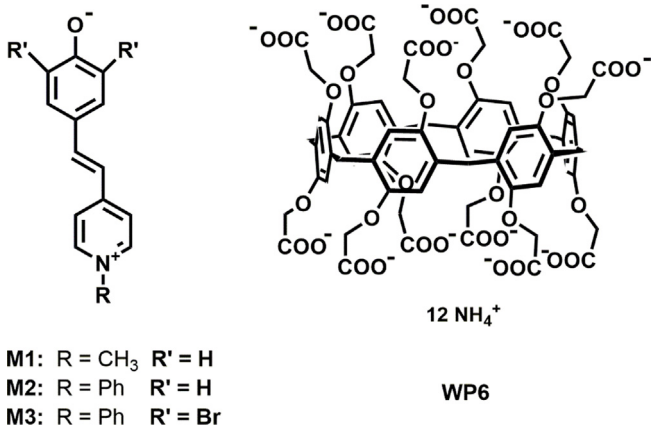


**Fig. 2.** The structures of the merocyanine dye guests and the pillararene host.

[1]. The absorption band of M1 appears at 442 nm in water and it shifts to 620 nm in $CHCl_3$ [2]. This negative solvatochromism of the dye (hypsochromic shift with increasing solvent polarity) shown in the majority of solvents, reverses in solvents of low polarity [3]. In the generally accepted interpretation of the solvatochromic properties of M1, the structure of the dye is described as a hybrid of a zwitterionic benzenoid and a neutral quinonoid canonical structure. In weakly polar solvents the hybrid is weighted toward the neutral form, in polar solvents the zwitterionic form is dominant. Theoretical calculations provided insightful information on how solute–solvent interactions affect the molecular geometry, the charge distribution and the $S_0 \rightarrow S_1$ excitation energy of the M1 dye solute [4–6]. Exploiting the high sensitivity of M1 to the local environment, it is frequently applied as a solvatochromic probe in liquid structure studies [7–9].

With its aromatic rings and oppositely charged ends, zwitterionic M1 can coordinate to macrocyclic receptors in multiple ways. So far, the complexes of M1 with cyclodextrins (CD-s), cucurbit[8]uril (CB8) and a calix[4]pyrrol (CP) have been described. α-, β-, γ- and methyl-β-CD form 1:1 inclusion complexes with M1 [10–12]. The complex of M1 with β-CD is more stable than its complexes with α-and γ-CD. As a consequence of the higher stability, the trans–cis photoisomerization of the protonated form of M1 is hindered in the β-CD complex of the dye [12,13]. CB8 accommodates two M1 molecules which are aligned head-to-tail in its cavity and which dimerize in a photochemical reaction [14,15]. The M1-CP complex is held together by hydrogen bonds between the phenolate oxygen atom of M1 and the pyrrol NH groups of CP [16]. In acetonitrile, this complex works as a colorimetric indicator displacement assay for the basic anions $F^-$, $Cl^-$ and $H_2PO_4^-$.

In the present work, the complexes of three merocyanines, M1-M3 in Fig. 2 with the water soluble pillararene macrocycle WP6 were studied. M2 and M3 were new compounds. Our final goal was to construct an indicator displacement assay (IDA) for the detection of cationic biomolecules, exploiting the high affinity of the multiple negatively charged carboxylato pillararenes to bind positively charged organic species [17–30].

The protic equilibria between the cationic phenol and the zwitterionic phenolate forms of the merocyanines was an important aspect of the design. The p$K_a$ of the phenol form of M1 is 8.57 [31]. Thus, the phenolate form of M1, the spectral properties of which are more sensitive to complexation, is dominant only in basic media - a disadvantage with respect to potential applications in biological samples. The phenyl substituent in M2 was expected to modify the binding constant with WP6 host, but not the acidity of the dye. In contrast, the introduction of the two bromo substituents in the phenol ring, was expected to lower the p$K_a$ significantly [32], for which zwitterion M3 was hoped to form a stable complex with WP6 under neutral conditions. As a part of this work, the p$K_a$ values of the free merocyanines and their WP6 complexes were determined in spectroscopic experiments and the results confirmed this hypothesis. Therefore, the M3–WP6 complex was chosen to be tested as a displacement assay. The performance of the assay was tested on the discrimination of lysine and its methylated derivatives.

## 2. Materials and methods

### 2.1. Synthesis

The pillararene host WP6 was synthesized using the method of Huang et al. [33]. Brooker's merocyanine M1 was prepared from 1-methylpicolinium iodide and 4-hydroxybenzaldehyde by Knoevenagel condensation, as described in Ref [34]. The two new merocyanine dyes, M2 and M3, were also prepared by Knoevenagel condensation reactions, from the corresponding *N*-substituted picolinium salt and benzaldehyde. The starting materials used in the syntheses were commercial products. The NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer. The exact mass measurements were performed with a Waters Q-TOF Premier mass spectrometer using electrospray ionization in positive mode.

#### 2.1.1. (E)-4-(2-(1-phenylpyridin-1-ium-4-yl)vinyl)phenolate (M2)

To a solution of 1-phenylpicolinium chloride (411 mg, 2.0 mmol) and 4-hydroxybenzaldehyde (305 mg, 2.5 mmol, 1.25 equiv) in 15 mL methanol was added a few drops of piperidine. The reaction mixture was refluxed overnight then the solvent was evaporated. The crude product was crystallized from a mixture of 10 mL acetone and 10 mL diethyl ether, filtered and washed thoroughly with acetone and ether. The formed chloride salt (phenol form) was suspended in 5 mL water, then an equivalent amount of 1 mol/L NaOH solution was added (1.56 mL based on the chloride salt). The brown suspension became deep red and was filtered, washed three times with cold water and dried to yield 423 mg (77%) purple crystalline solid. M.p. 230–232 °C.

$^1$H NMR (500 MHz, DMSO $d_6$): $\delta$ 8.91 (d, $J$ = 5.8 Hz, 2H), 8.07–8.01 (m, 3H), 7.80 (d, $J$ = 7.3 Hz, 2H), 7.69–7.65 (m, 3H), 7.58 (d, $J$ = 8.2 Hz, 2H), 7.18 (d, $J$ = 15.9 Hz, 1H), 6.70 (d, $J$ = 8.2 Hz, 2H).

$^{13}$C NMR (126 MHz, DMSO $d_6$, TFA was added to the solution to increase solubility): 160.55, 154.63, 143.75, 142.94, 142.44, 130.85, 130.74, 130.25, 126.43, 124.43, 123.13, 119.60, 116.23.

HR-MS calculated mass for $C_{19}H_{16}NO$ $[M + H]^+$ $m/z$ = 274.1232, found 274.1236.

*2.1.2. (E)-2,6-dibromo-4-(2-(1-phenylpyridin-1-ium-4-yl)vinyl) phenolate (M3)*

To a solution of 1-phenylpicolinium chloride (250 mg, 1.21 mmol) and 3,5-dibromo-4-hydroxybenzaldehyde (508 mg, 1.82 mmol, 1.5 equiv) in 10 mL methanol was added a few drops of piperidine. The reaction mixture was refluxed overnight then the solvent was evaporated. The crude product was crystallized from 20 mL acetone, filtered and washed thoroughly with acetone. The formed chloride salt (phenol form) was suspended in 5 mL water, then an equivalent amount of 1 M NaOH solution was added (1.06 mL based on the chloride salt). The brown suspension became deep red and was filtered, washed three times with cold water and dried to yield 453 mg (87%) purple crystalline solid. M.p. 256–258 °C.

$^1$H NMR (500 MHz, DMSO $d_6$): $\delta$ 8.70 (d, $J$ = 6.8 Hz, 2H), 7.88 (d, $J$ = 15.3 Hz, 1H), 7.82–7.77 (m, 6H), 7.68 (t, $J$ = 6.8 Hz, 2H), 7.63 (t, $J$ = 7.0 Hz, 1H), 6.88 (d, $J$ = 15.3 Hz, 1H).

$^{13}$C NMR (126 MHz, DMSO $d_6$, TFA was added to the solution to increase solubility): $\delta$ 153.71, 152.68, 144.22, 142.41, 139.15, 132.22, 130.95, 130.22, 129.86, 124.43, 123.59, 122.95, 112.31.

HR-MS calculated mass for $C_{19}H_{14}NOBr_2$ $[M + H]^+$ $m/z$ = 429.9442, found 429.9446.

*2.2. UV–VIS spectra of merocyanine dyes and their WP6 complexes*

The samples for these experiments were solutions of the pure dyes in Britton Robinson buffers and solutions of dye-WP6 mixtures in Britton Robinson buffers and in 0.2 mol/L aqueous NaOH. The ionic strengths of the buffers were adjusted to 0.2 mol/L with appropriate amounts of KCl [35]. The spectra were recorded on an Agilent 8453 diode array UV–VIS absorption spectrometer. All the experiments were carried out at 25 °C.

*2.3. Calculation of equilibrium constants*

The equilibrium constants (the acidic dissociation constants of the pure dyes and their WP6 complexes and the binding constants of the dye-WP6 complexes) were determined by a least square fitting to the absorption spectra of dye solutions measured at different pH values or at different pillararene concentrations. The dye formed four absorbing species, the free phenol $M^+$, the free phenolate M (this corresponds to the zwitterion), the complexed phenol and the complexed phenolate. The absorbance values of the samples at a selected wavelength $\lambda$, were related to the absorption coefficients of the four species $\varepsilon^{\lambda}_{M}, \varepsilon^{\lambda}_{MH^+}, \varepsilon^{\lambda}_{M\cdot WP6}, \varepsilon^{\lambda}_{MH^+\cdot WP6}$, the total concentrations, $c^0_M$ and $c^0_{WP6}$, the pH, in addition to the respective equilibrium constant. Such expressions were derived starting from Beer's law, the mass balance equations and the equations of the equilibrium constants [36]. A detailed description of the methods used for the determination of the equilibrium constants is provided in Section S2 of Supplementary Materials (SM).

## 3. Results and discussion

*3.1. Synthesis of merocyanine dyes*

The merocyanine dyes M1- M3 were synthesized as outlined in Fig. 3. The synthesis of M1 (Brooker's merocyanine) was performed as described in Ref. [34]. The new compounds M2 and M3 were prepared from 1-phenylpicolinium chloride [37] and 4-hydroxybenzaldehyde or 3,5-dibromo-4-hydroxybenzaldehyde, respectively. First, the phenol forms of the dyes were prepared by the piperidine-catalyzed Knoevenagel condensation. The phenolate forms (inner salts) were obtained then by treating the aqueous suspensions of the phenolate form dyes by NaOH.

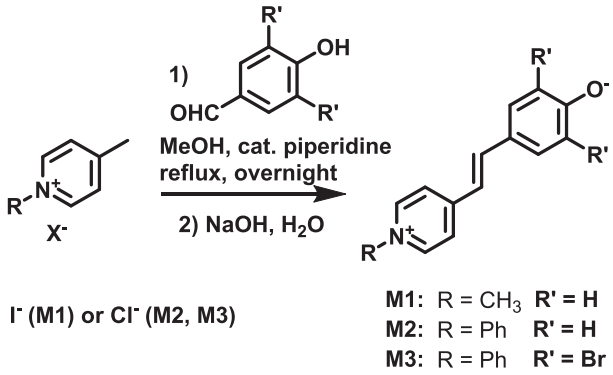


**Fig. 3.** Scheme of synthesis of merocyanine dyes M1-M3.

*3.2. Merocyanine dye – WP6 systems*

*3.2.1. Reaction scheme*

The properties of the merocyanine dye – pillararene systems were described in terms of four equilibrium reactions (see Fig. 4):

(i) the acidic dissociation of the cationic pyridinium phenol dye producing the phenolate (i.e. the zwitterionic) species;
(ii) the binding of the phenol form dye guest to the to the pillararene host;
(iii) the binding of the phenolate (zwitterionic) dye guest to the to the pillararene host;
(iv) the acidic dissociation of the complex of the phenol form dye with the pillararene host.

The 1:1 stoichiometry of the M−WP6 complexes was confirmed by the Job plots of the absorbance data of the three dye-pillararene systems (see Figs. S7-S9).

We note that WP6 was considered fully dissociated in the dye-WP6 mixtures since only samples of pH $\geq$ 6.5 were used. In more acidic (pH 6.0 in Ref. [19]) solutions, partially dissociated WP6 appears which precipitates [33].

As shown in Fig. 4, reactions (i)-(iv) constitute a cyclic scheme. In principle, any of the four equilibrium constants in the cycle can be obtained from the other three $K$ values. The accuracy of an indirectly determined equilibrium constant is, however, limited by the error propagation. Therefore, we attempted to determine the four equilibrium constants in separate experiments.

The values of the equilibrium constants obtained from the experimental spectra are presented in Table 1.

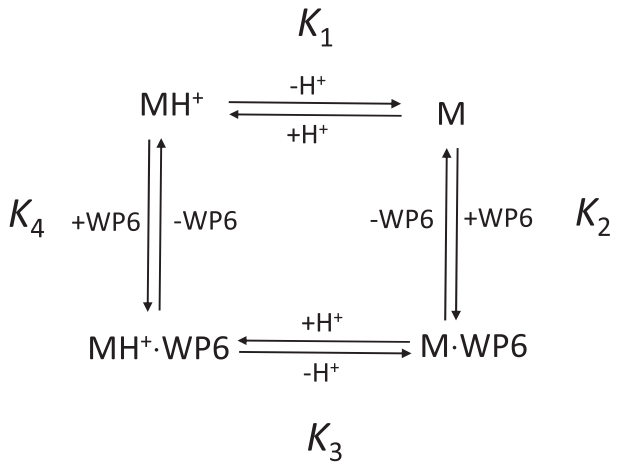


**Fig. 4.** Scheme of reactions in merocyanine dye M – pillararene WP6 systems.

**Table 1**
Equilibrium constants $K_1$ – $K_4$ for the reactions in merocyanine dye – WP6 mixtures.[a]

| System | Acidic dissociation constants | | Binding constants ($Lmol^{-1}$) | |
|---|---|---|---|---|
| | $-logK_1$ | $-logK_3$ | $K_2$ | $K_4$ |
| M1 – WP6 | 8.66, (8.57)[b] | 10.23 | $1.24{\cdot}10^5$ | $1.26{\cdot}10^6$ |
| M2 – WP6 | 8.44 | 9.51 | $4.90{\cdot}10^6$ | $5.25\cdot10^7$ |
| M3 – WP6 | 4.79 | 5.99 | $1.05{\cdot}10^5$ | $1.66{\cdot}10^6$ |

[a] This work, determined in Britton-Robinson buffers with ionic strength of 0.2 mol/L.
[b] Ref. [31].

### 3.2.2. Acidic dissociation constants

First, the dissociation constants $K_1$ for the phenol forms of M1-M3 were determined from their absorption spectra measured as function of pH. The results are illustrated in Fig. 5a showing the spectra of M3. (The pH dependent spectra of all the three merocyanine dyes are presented in Fig. S10 in SM). As can be seen in Table 1, the $pK_a$ value of $M2H^+$ was very close to the $pK_a$ of $M1H^+$, the latter reported also in Ref.[31] In contrast, with the two bromines in the phenol part of M3, the desired enhancement of the acidity was achieved – M3 was present in its zwitterionic form in neutral solutions.

The $K_3$ equilibrium constants, corresponding to the protic equilibria of the complexed dyes, were determined from the pH dependence of the spectra of samples containing WP6 in large excesses over the initial concentrations of the dyes. As example, the pH dependent spectra of the M3-WP6 system is shown in see Fig. 5b. The variation of the spectra of all the three M-WP6 systems with the pH can be seen in Fig. S11 in SM. The presence of isobestic points in the spectra confirmed that the spectra were the linear combinations of the spectra of two absorbing components, M·WP6 and $MH^+$·WP6, the contributions of the uncomplexed forms of the dye, M and $MH^+$ were negligible.

The spectral changes in the M1-WP6 and M2-WP6 systems could be followed in the full range of the acid-base equilibrium, allowing an accurate determination of the $K_3$ equilibrium constants, using $K_3$ as the only fitting parameter. In case of the mixtures of the more acidic M3 with WP6, the pure protonated complex could not be approached up to the pH limit of 6.0. In this case, the absorption coefficients of the complex in the range of its absorption band were also treated as fitting parameters.

Comparing the $pK_a$ values for the free and complexed merocyanines in Table 1, it can be seen that the acidity of all the three merocyanines decreased substantially in the complexes. Presumably, the increase of $pK_a$ is a combined effect of the reduced polarity of the local environment of the dyes in the pillararene cavity and the electrostatic field of the carboxylate groups of the WP6

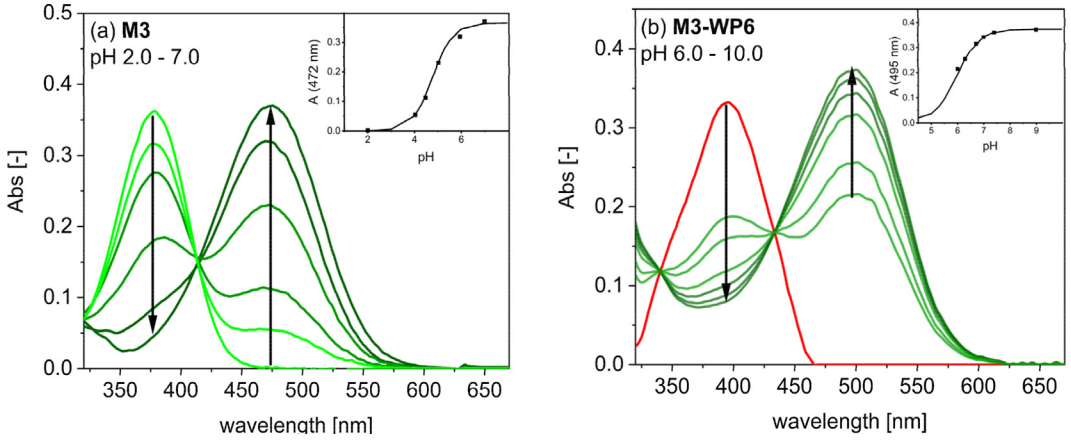


**Fig. 5.** pH dependent spectra of (a) $1.0{\cdot}10^{-5}$ M solution of M3 and (b) a mixture of $1.0{\cdot}10^{-5}$ mol/L M3 and $2.0{\cdot}10^{-4}$ mol/L WP6. The red trace is the spectrum of complex $M3H^+$·WP6 obtained by least-squares fitting. The insets show the plots of the absorbance values at the band maxima of (a) merocyanine M3 and (b) its WP6 complex versus the pH of the samples.

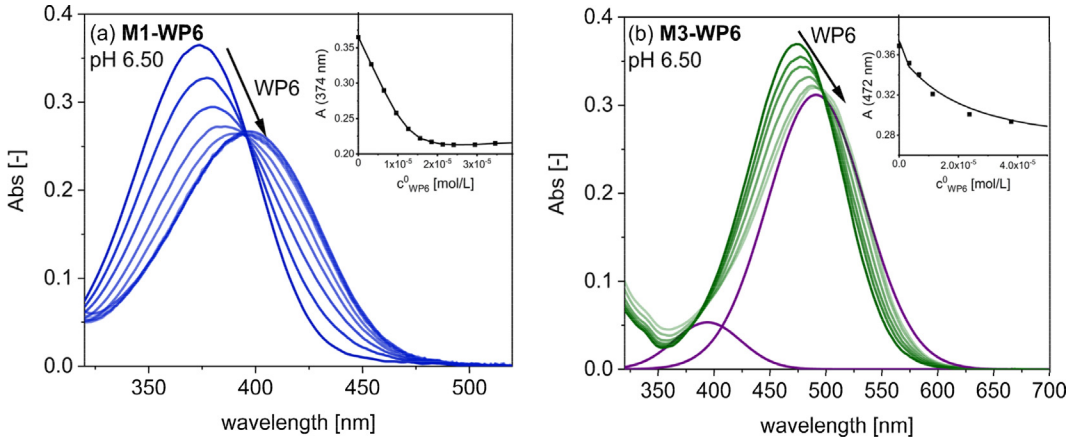


**Fig. 6.** Absorption spectra of (a) M1-WP6 and (b) M3 – WP6 mixtures in pH 6.50 solutions. Total concentrations (a) $c^0_{M1}$ = $1.0{\cdot}10^{-5}$ mol/L, $c^0_{WP6}$ = $0–4{\cdot}10^{-5}$ mol/L; (b) $c^0_{M3}$ = $1.0{\cdot}10^{-5}$ mol/L, $c^0_{WP6}$ = $0–5{\cdot}10^{-5}$ mol/L. The violet traces in (b) show the contributions of M3·WP6 and $M3H^+$·WP6 to the spectra of the sample with $c^0_{WP6}$ = $5{\cdot}10^{-5}$ mol/L. The insets show the plots of the absorbance values at the band maxima of (a) $M1H^+$ and (b) M3 versus the total concentrations of WP6.

host. The p$K_a$ shifts of acid-base indicators in micelles of charged surfactants has been evaluated in terms of the above combined effect [38,39].

*3.2.3. Spectra of merocyanine dye – WP6 complexes*

The complexation of the three merocyanine dyes were studied on the spectra of mixtures with the same dye and different WP6 concentrations. First, two sets of spectra were recorded for each dye-WP6 system, one set at pH 6.5 (see Fig. 6), the other set at pH 10.5 (see Fig. 7). As mentioned above, the dissociation of WP6 can be taken complete only in pH > 6.0 solutions.

As follows from their p$K_a$ values, at pH 6.5 the uncomplexed M1 and M2 dyes are present dominantly in their undissociated phenol form, whereas M3 is present in its phenolate form. Adding WP6 to the solution of M1, a new band emerged at higher wavelength (see Fig. 6a). This band belongs to the complexed phenol, $M1H^+$·WP6. The spectra of the $M2H^+$ - WP6 mixtures showed similar changes (Fig. S12b).

The addition of WP6 to the pH 6.5 solution of M3 resulted in the appearance of two new features (see the resolved spectrum in Fig. 6b). A comparison of the spectra to the spectra of M3 at various pH-s, in the presence of WP6 in large excess (Fig. 5b), made it clear that the new bands belonged to the complexes of the phenolate and phenol forms of the dye, M3·WP6 and $M3H^+$·WP6. The formation of the phenol complex from the phenolate guest was an additional evidence that the acidity of M3 was reduced significantly on the coordination by WP6.

The spectra of the M1-WP6 systems, measured at pH = 10.5 (see Fig. 7a) contained also the contributions of three absorbing components, like the spectra of M3-WP6 systems measured at pH 6.5 (see Fig. 6b). These spectra proved that the reaction of the deprotonated dye, M1 with WP6 yielded a mixture of the complexes with protonated and deprotonated dye guests, $M1H^+$·WP6 and M1·WP6. Similarly, in the M2 – WP6 mixtures both $M2H^+$·WP6 and M2·WP6 were formed. In contrast, the phenolate form of the stronger acidic dye, M3 formed only one type of complex, M3·WP6 with the pillararene host in these basic solutions, no proton-uptake was indicated by the spectra in Fig. 7b.

The spectra of the M1-WP6 and M2-WP6 systems were also measured in 0.2 M NaOH solutions (see Fig. S13). These spectra were the linear combinations of the spectra of the phenolate form dyes and their WP6 complexes, the bands of the of the phenol form merocyanine – WP6 complexes were not observable in these strongly basic solutions.

The spectral data of the dyes in the absence of WP6 and at high WP6 concentrations where the complexation is close to complete, are compared in Table 2. Both in the cases of the phenol and phenolate form dyes, the complexation results in 20–40 nm shifts of the absorption bands to higher wavelengths. The shifts of the zwitterionic (phenolate) forms are accordance with the lower local polarity in the cavity of the WP6 host compared to the polarity in bulk water. The visible band of M1 also shifts to the red in the spectra of its cyclodextrin complexes [10,11,12]. The solvatochromism of the phenol forms of Brooker merocyanines have not been analyzed yet in detail. A similar red shift has been observed in the spectrum of DASPI (the pyridinium styryl dye with a dimethylamino substituent in the place of the OH group of $M1H^+$) in the presence of WP6 [27].

*3.2.4. Binding constants*

The binding constants $K_2$ were determined by a one parameter fitting to the spectra of the three dyes in basic solutions, in the presence of WP6 in different concentrations. In case of M3·WP6, the spectra measured in pH 10.5 buffers (Fig. 7b) were used as experimental data. In cases of the complexes M1·WP6 and M2·WP6, the p$K_a$-s of which fall in the basic range, the values of $K_2$ were obtained from spectra measured in 0.2 mol/L NaOH solutions (see Figs. S13a-b in the SM).

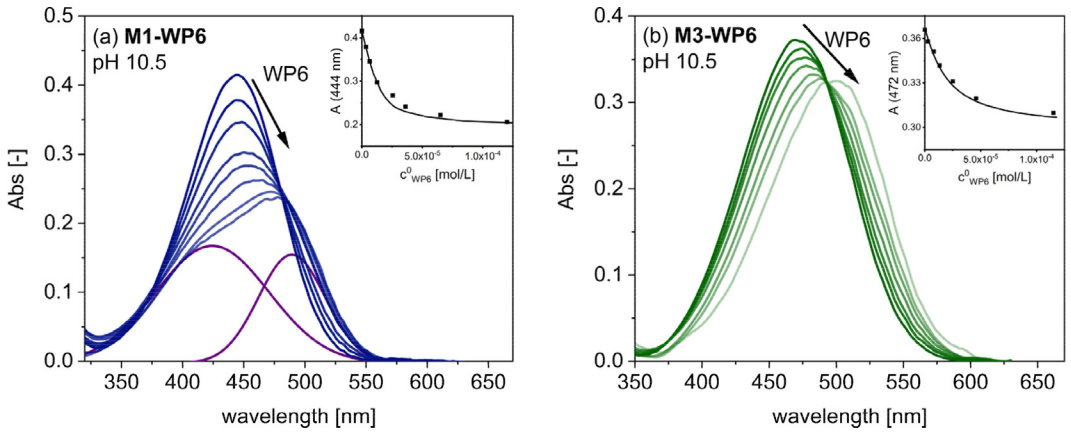


**Fig. 7.** Absorption spectra of (a) M1-WP6 and (b) M3 – WP6 mixtures in pH 10.5 solutions. Total concentrations are (a) $c^0_{M1} = 1.0{\cdot}10^{-5}$ M, $c^0_{WP6} = 0–1.2{\cdot}10^{-4}$ mol/L; (b) $c^0_{M3} = 1.0{\cdot}10^{-5}$ mol/L, $c^0_{WP6} = 0–1.2{\cdot}10^{-4}$ mol/L. The violet traces in (a) show the contributions of M1·WP6 and $M1H^+$·WP6 to the spectra of the sample with $c^0_{WP6} = 1.2{\cdot}10^{-4}$ mol/L. The insets show the plots of the absorbance values at the band maxima of (a) M1 and (b) M3 versus the total concentration of WP6.

**Table 2**
Absorption spectral data of neutral and protonated forms of merocyanine dyes M1-M3 and their WP6 complexes.

| | $MH^+$ | | M | | $MH^+$·WP6 | | M·WP6 | |
|---|---|---|---|---|---|---|---|---|
| | $\lambda_{max}$ [nm] | ε [$Lmol^{-1}cm^{-1}$] | $\lambda_{max}$ [nm] | ε [$Lmol^{-1}cm^{-1}$] | $\lambda_{max}$ [nm] | ε [$Lmol^{-1}cm^{-1}$] | $\lambda_{max}$ [nm] | ε [$Lmol^{-1}cm^{-1}$] |
| M1 | 374 | 36,500 | 444 | 42,800 | 396 | 26,700 | 479 | 36,500 |
| M2 | 394 | 35,500 | 475 | 35,600 | 415 | 29,500 | 506 | 30,500 |
| M3 | 377 | 36,700 | 472 | 37,000 | 394 | 33,100 | 495 | 37,100 |

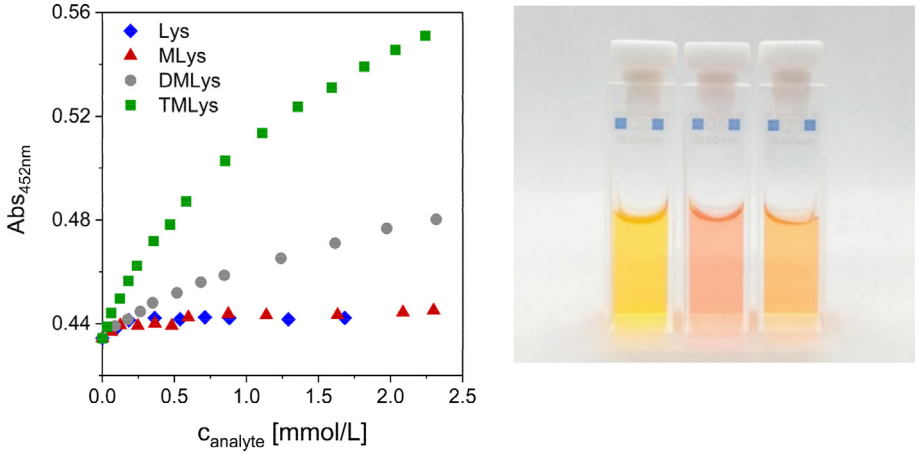


**Fig. 8.** Spectral changes of a M3–WP6 mixture ($c^0_{M3}$ = 2·10$^{-5}$ mol/L, $c^0_{WP6}$ = 6·10$^{-5}$ mol/L) on the addition of Lys and its methylated derivatives. (a) Absorbance values at 452 nm, (b) photographs of solutions of M3, M3 + WP6 and M3 + WP6 + Me3Lys ($c^0_{M3}$ = 2·10$^{-5}$ mol/L, $c^0_{WP6}$ = 6·10$^{-5}$ mol/L, $c^0_{Me_3Lys}$ = 2.2·10$^{-3}$ mol/L).

The values of $K_4$ for M1 and M2 were obtained in a similar manner, from the spectra of dye-WP6 mixtures with high WP6 excesses, measured in pH 6.5 buffers (Fig. 6a and S12b, the latter in the SM). In case of the stronger acidic dye M3, the binding constant $K_4$ could not be determined directly, since the undissociated pyridinium phenol form, M3H$^+$ is dominant only at low pH values where WP6 is not fully dissociated. The value of $K_4$ for M3 was estimated as $K_4 = (K_1 \cdot K_2)/K_3$.

Comparing the binding constants $K_2$ and $K_4$ in Table 1, it can be seen that the value of $K_4$ is higher by one order magnitude than the value of $K_2$ for the same dye. These differences are due to the strong electrostatic interactions between the positively charged MH$^+$ guests and the negatively charged WP6 host. The electrostatic interactions between the dye zwitterions and the WP6 host are weaker.

The highest $K_2$ value as well as the highest $K_4$ value belong to the complexes of M2. Presumably, the phenyl group on the pyridinium N atom intrudes deeply into the cavity of the WP6 hosts of the M2·WP6 and M2H$^+$·WP6 complexes. This way, these complexes are also stabilized by a strong hydrophobic effect.

Hydrophobic interactions must also contribute significantly to the stabilization of the complexes M3·WP6 and M3H$^+$·WP6 since the pyridinium group of M3 is also supplied with a phenyl substituent. The values of the $K_2$ and $K_4$ binding constants of the M3-WP6 complexes are lower than the respective values of the M2-WP6 complexes. This difference may arise from the better solvation of the dibrominated phenol moiety of M3 by water solvent molecules.

### 3.3. Indicator displacement

The phenol and the phenolate forms of the three merocyanines showed similar spectral shifts on complexation, the absorption band of the complexes were located at ~30 nm higher wavelengths than the bands of the uncomplexed guests. The advantages of the usage of a phenolate form dye as indicator for a displacement assay were that (i) the phenolates and their WP6 complexes absorbed in the visible range, thus, the complexation could be detected by naked eyes; (ii) their binding constants ($K_2$) were lower, than the binding constants of the phenol forms ($K_4$), making the displacement more efficient. The indicator M3 had the additional advantage of its low p$K_a$, the phenolate forms of the free and complexed indicator were dominant in neutral solutions. In contrast, in case of M1 and M2 this condition was met only in strongly basic media.

The colorimetric sensor in the displacement experiments was an M3-WP6 mixture with initial concentrations $c^0_{M3}$ = 2·10$^{-5}$ mol/L and $c^0_{WP6}$ = 6·10$^{-5}$ mol/L. The pH was set to 8.0. Calculated from the $K_2$ binding constant, 86% of the indicator was in complexed state in this mixture.

The variations of the spectra with the concentrations of Lys and its methylated derivatives are illustrated in Fig. 8. As can be seen in the figure, $Me_3Lys$ induced a strong change in the spectrum, the color change was visible even by naked eye. $Me_2Lys$ induced a weaker spectral response, whereas the effects of MeLys and Lys on the spectra were negligible.

The spectra of the M3 – WP6 – $Me_nLys$ mixtures afforded the calculation of the binding constants for the WP6 complexes of $Me_3Lys$ and $Me_2Lys$. The compositions of the displacement assays were governed by the simultaneous reactions

$$M + WP6 \rightleftarrows M \cdot WP6, \text{ with equilibrium constant } K_2$$

and

$$A + WP6 \rightleftarrows A \cdot WP6, \text{ with equilibrium constant } K_5,$$

where A denotes the $Me_3Lys$ or $Me_2Lys$ analyte.

The value of $K_2$ was obtained earlier from the spectra of binary M3 –WP6 systems. A fitting to the spectra of the M3 –WP6 – $Me_3$-Lys and M3 –WP6 – $Me_2Lys$ ternary systems yielded $K_5 = 2.7 \cdot 10^3$ Lmol$^{-1}$ for $Me_3Lys$·WP6 and $6.6 \cdot 10^2$ Lmol$^{-1}$ for $Me_2Lys$·WP6. It is worth to note that WP5, the smaller ring-sized analogue of WP6, forms a complex of higher stability with Lys than with $Me_3Lys$ [40]. This reverse order of the affinities is an interesting example of size-selective complexation in supramolecular chemistry.

## 4. Conclusions

Our work on the complexes of the pyridinium phenolate dyes M1-M3 with carboxylato pillar[6]arene WP6 showed that the dyes occur in four forms in the systems which are the undissociated cationic phenol, MH$^+$ and its complex, MH$^+$·WP6 and the zwitterionic phenolate form, M and its complex, M·WP6. The two acidic dissociation constants and the two binding constants governing the compositions of the systems were determined from the absorption spectra. The absorption bands of the zwitterionic forms showed a large batochromic shift on complexation, making M1-M3 suitable indicators for WP6-based indicator displacement systems. Comparing the p$K_a$-s and the binding constants of the three dyes, the dibromo derivative M3 was selected as an indicator of such sensing system for the detection of cationic biomolecules. The

M3-WP6 complex was found an effective assay for the detection of $Me_3Lys$. As the $pK_a$ values of pyridinium phenolate dyes increase much on complexation, the binding of the free phenol forms of M1-M3 and analogous dyes to WP6 leads to the complexes of the phenolate forms in the appropriate pH interval, providing further perspectives for the design of pillararene-based chemosensors.

## CRediT authorship contribution statement

**Dóra Hessz:** Investigation, Methodology, Formal analysis. **Stella Bádogos:** Investigation. **Márton Bojtár:** Investigation, Writing - original draft. **István Bitter:** Conceptualization. **László Drahos:** Investigation. **Miklós Kubinyi:** Project administration, Funding acquisition, Conceptualization, Writing - original draft, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgement

The research reported in this paper and carried out at BME has been supported by the NRDI Fund (TKP2020 IES, Grant BME-IE-BIO) based on the charter of bolster issued by the NRDI Office under the auspices of the Hungarian Ministry for Innovation and Technology.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2021.119455.

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