Reversal of metachromasy revisited; displacement of Toluidine-blue from alginate by surfactants

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HIGHLIGHTS

- The metachromatic alginate-TBO complex has a 1:1 charge stoichiometry.
- Reversal of metachromasy, is charge stoichiometric for cationic surfactant.
- It is not generic, but depends on surfactant structure, for anionic surfactants.
- The ease of forming premicellar structures determines ease of reversal.
- Efficiency of SLES can be ranked as: (EO)\textsubscript{2} > (EO)\textsubscript{4} > (EO)\textsubscript{8} > (EO)\textsubscript{12} > (EO)\textsubscript{30}.

GRAPHICAL ABSTRACT

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ABSTRACT

The color stability of dyes is crucial in commercial dyeing, whereas their use in scientific research often depends on color changes due to metachromasy. This is the result self-organization of dye molecules into a stacked organization onto a charged template, such as an oppositely charged polyelectrolyte. Literature shows that ‘reversal of metachromasy’, caused by the dye’s displacement from its stacked state, can be induced by a.o. surfactants. This is suggested to be a generic effect. We investigated the reversal of metachromasy using the alginate-ortho toluidine blue (TBO) metachromatic complex in a low ionic strength buffer system (phosphate 1 mM, pH = 7). Under these conditions alginate has an ‘apparent’ pKa of 4.6 and the alginate-TBO complex shows to be 1:1 charge stoichiometric. Displacement by the cationic surfactant TEGO’\textsuperscript{trant} A100 (1,3-Didecyl-2-methylimidazolium chloride, TEGO) is found to be charge stoichiometric, its pathway depending on initial complex composition. TEGO initially binds to free binding sites on alginate prior to its competition with TBO for which it arranges into similar stacks allowing cooperative binding.

Further, the effect of offering TBO alternative binding sites is studied by adding negatively charged surfactants. Binding of TBO to anionic surfactants, was found to depend on the ease of these to form premicellar structures which offer an alternative binding motive for TBO. Premicellar aggregation depends on the surfactant’s structure; i.e. size of the ethylene oxide (EO) block, within a homologous series of sodium lauryl ether sulfates (SLES) and from our experiments can be ranked as (EO)\textsubscript{2} > (EO)\textsubscript{4} > (EO)\textsubscript{8} > (EO)\textsubscript{12} > (EO)\textsubscript{30}.

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Reversal of metachromasy by surfactants, although proceeding via a similar mechanism, is not generic but depends on surfactant structure and its chemical composition.

1. Introduction

Dyes, such as the cationic dye ortho-Toluidine blue (TBO) used in this study, have an increasingly wide range of applications which extends beyond their original use as coloration agents [1,2]. Next to the traditional use in histopathology, textiles dyeing and printing inks, they find abundant application in chemical research such as analytical chemistry [3,4], biochemistry and in sensing molecular interactions [5,6]. Besides these valuable functions of dyes, their abundant industrial use and inherent toxicity also poses an environmental risk [7,8]. In coloration applications a stable color performance is important whilst in chemical research often a color change, depending on the binding or aggregation state of the dye, is desired. Such a distinct spectral shift is called metachromasy [9] and results from the dye organizing itself in aggregates or stacks, which is typical for many dyes including TBO. Metachromasy can be observed when the dye binds electrostatically to an oppositely charged template or guiding motive in such manner that the dye ions form discrete stacks or aggregates. This motive can be a polyelectrolyte [10,11], an assembly of surfactants [12,13] or a charged surface [14]. In textiles dyeing, where polyelectrolytes or surfactant may be used to promote dye binding to the substrate, metachromasy is undesired. In contrast, metachromasy can be crucial in its role to detect molecular interactions.

An example of a motive is alginate, a hydrophilic, natural copolymer consisting of α-L-guluronic acid (G) and β-D-mannuronic acid (M) monomeric units organized partly in homo-polymeric G or M blocks and partly in alternating MG blocks [15]. Alginate is mostly known for its use as thickening agent in the food industry, but next to that also as a template for the binding of dyes [17,18], whereas literature on its suitability as a template for TBO complexation is sparse [19] with a variation of the ethylene oxide (EO) block size. From this we aim at obtaining information on the interaction strength between TBO and alginate and/or alternatively between TBO and premicellar structures of the SLES surfactants as a function of their EO-block size.

2. Materials and methods

2.1. Materials

Ortho-toluidine blue (TBO), (7-amino-8-methyl-phenothiazin-3-ylidene)-dimethyl-ammonium chloride, analytical grade, was obtained from Fluka Analytical (India). Alginate, alginic acid sodium salt from brown algae, was purchased from Sigma Aldrich (# 71238). The homologous series of sodium lauryl ether sulfates (SLES) used were commercial samples of industrial grade products, supplied by BASF, of Disponil® series surfactants: (1) Disponil FES 27; Sodium lauryl (EO)2 sulfate, (2) Disponil FES 32; Sodium lauryl (EO)3 sulfate (3) Disponil FES 993; Sodium lauryl (EO)12 sulfate and (4) FES 77; Sodium lauryl (EO)30 sulfate. Sodium Dodecyl Sulfate of highest purity (99.6%) available, was purchased from Merck (Germany). TEGO® trant A100, or 1,3-didecyl-2-methylimidazolium chloride (DDMICl, further abbreviated as TEGO), which is presumed to directly compete with TBO for a binding site on alginate. The displacement by anionic surfactants will be the result of offering TBO an alternative binding site. As anionic surfactants we employ, a, previously used, homologous series of SLES [19] with a variation of the ethylene oxide (EO) block size. From this displaceability would occur in a similar manner, regardless of surfactant charge, structure and composition. As a model system we have chosen the alginate-TBO complex for which we firstly evaluate alginate’s suitability as a template for TBO complexation under well-defined conditions of pH and ionic strength. This includes a determination of the apparent pKₐ under applied experimental conditions, as well as establishing the binding stoichiometry of the alginate-TBO complex. Next, alginate-TBO complexes with pre-defined composition are used to study the displacement of TBO by cationic and anionic surfactants. As a model cationic surfactant we have chosen TEGO® trant A100: 1,3-didecyl-2-methylimidazolium chloride (DDMICl, further abbreviated as TEGO), which is presumed to directly compete with TBO for a binding site on alginate. The displacement by anionic surfactants will be the result of offering TBO an alternative binding site. As anionic surfactants we employ, a, previously used, homologous series of SLES [19] with a variation of the ethylene oxide (EO) block size. From this we aim at obtaining information on the interaction strength between TBO and alginate and/or alternatively between TBO and premicellar structures of the SLES surfactants as a function of their EO-block size.
hydrochloride (PDADMAC) 1 mM standardized titrant solution was purchased from BTG (Switzerland). All other chemicals were of highest purity available. All materials were used as received without further purification. Ultrapure water, used throughout the investigation, was obtained using a Milli-Q Advantage A10 purification system from Millipore SA Molsheim (France). Scheme 1 shows the generic molecular structures of Alginic acid, TBO, TEGO® trant A100, SDS and SLES.

2.2. Methods

2.2.1. Reagent preparation

All reagents were prepared as stock solutions, at a suitable concentration, in a phosphate buffer at c(buffer) = 1 mM and pH = 7.0. The stock solutions of alginate, SDS and SLES were prepared at 10 mM (the experimental verification thereof is described in the next sub-section) and TBO at 1 mM concentration. In all experiments a constant pH and ionic strength was maintained by further diluting the reagents in the same phosphate buffer (c(buffer) = 1 mM and pH = 7.0).

2.2.2. Determination and definition of polymer concentration (P) and surfactant concentration (S)

The alginate material used in this study is not a pure compound, as can be judged by supplier’s indicated range in loss on drying (≤15%). As alginate is envisaged as a template for the electrostatic binding of TBO, its ‘functional’ concentration P was defined as the equivalent charge density (expressed as mM) of ionized groups at pH = 7. The quantity P was determined by streaming potential titration [20,21], employing a Mütek™ PCD-05 Particle Charge Detector fitted with a standardized PDADMAC titrant solution, of the alginate stock solution. To ensure a constant pH, titration was performed in phosphate buffer (C(buffer) = 10 mM, pH = 7) and consisted of quadruple analyses which were averaged to obtain the functional alginate concentration P (data shown in Supporting information, from here on referred to as SI).

The surfactants used in this study were, apart from SDS, industrial grade commercial SLES samples. The generic molecular formula for these products represents an average composition, as there is both distribution in the alkyl-chain length and in the number of EO-groups per molecule. Partial hydrolysis of the sulfate-ether bond, e.g. during synthesis, causes also species to be present that do not carry a sulfate group. Furthermore the SLES surfactants were supplied as an aqueous solution with only an indicated range of active matter, expressed as weight fraction (wt%). Likewise, as we intended to study the effect of, mainly, electrostatic interactions between oppositely charged species, we defined surfactant concentration as moles per liter of charged surfactant species, regardless of their actual chemical composition and structure. In a previous study [22] it was shown that this is feasible by direct aqueous one-phase titration [23] using the cationic titrant TEGO® trant A100 [24,25]. To achieve this, we applied monomolecular turbidimetric titration employing a Metrohm 907 Titrando titrator fitted with a Metrohm SpectroSense photometric detector (wavelength \( \lambda = 610 \) nm) and a freshly prepared, and standardized, 4.0 mM TEGO® trant A100 (DDMCl) titrant solution [6]. The SLES surfactants were analyzed in triplicate, yielding the average SLES content in the original samples. Based on the SLES content thus found (given in SI), stock solutions were made in which the SLES concentration S was defined as the amount that followed from titration using TEGO® trant A100 (TEGO). Verification of the actual SLES concentration S in the final stock solutions, using the same method, showed that all were at their targeted value (within 2%, the accuracy of the applied method).

2.2.3. Determination of the ‘apparent’ \( pK_a \) of alginate under experimental conditions

To determine the ‘apparent’ or effective \( pK_a \) of alginate, under the experimental conditions used in this study, two independent methods were employed: potentiometric titration [26] and surface tension measurement [27]. Potentiometric titration was performed with a Metrohm 907 Titrando titrator fitted with a calibrated glass electrode. First a 10-fold diluted aliquot (dilution medium: 1 mM phosphate buffer \( pH = 7 \)) of the alginate stock solution was titrated with a standardized 0.01 M potassium hydroxide (KOH) solution to \( pH = 9 \), next this solution was back-titrated using a standardized 0.01 M nitric acid \( (HNO_3) \) solution. The equivalence point obtained in the forward titration was used to calculate the excess KOH added, which was subsequently subtracted from the volumetric addition of \( HNO_3 \) data in the back-titration. The \( pK_a \) value was found at the \( pH \)-value at 1.5 times the equivalence point in the corrected back-titration curve. During the titration, calculated from the added titrant volume and the volume of the titration mixture, the ionic strength increased in the same order of magnitude as that of the buffer present, which was assumed not to have a significant effect on the results. Quadruple titrations were performed and the resulting \( pK_a \) values averaged. The determination of the ‘apparent’ \( pK_a \) values of polyelectrolytes, by measuring the surface tension at the air-polyelectrolyte solution interface as a function of \( pH \), was recently reported [27]. This methodology was applied, employing an Attension Sigma-70 tensiometer using the Wilhelmy-plate method. 40 g of alginate stock solution \( (C(alginate) = 10 mM) \) in phosphate buffer \( (C(buffer) = 1 mM, \ pH = 7.0) \) was pipetted into a thoroughly cleaned glass cup. After the \( pH \) of this solution was measured, using a Knick 761 Calimatic \( pH \)-meter fitted with a Schott Blueline 16 pH micro \( pH \)-electrode, the surface tension was determined in triplicate and the results were averaged. Next, the \( pH \) of the solution was stepwise lowered by volumetric addition of 0.1 M \( HNO_3 \) solution using calibrated micropipettes. After each \( HNO_3 \) addition, the solution was briefly mixed until a stable \( pH \)-value was attained after which the \( pH \) was registered and the surface tension measured as described. During the titration, the ionic strength increased due to the addition of titrant (additional salt resulting from titrant estimated at 5 mM), also this increase was assumed to have negligible effect on the results. The apparent \( pK_a \) was obtained from the inflection point \( (X_0) \) in a sigmoidal or Boltzmann-fit of surface tension values plotted against solution \( pH \).

2.2.4. Spectral studies on alginate-TBO interactions and displacement studies

The study of interactions between alginate and TBO, and its displacement by surfactants, was done by recording visible light absorbance (wavelength \( \lambda \) scan from 800 to 350 nm) of solutions by UV–vis spectrophotometry, employing a Shimadzu UV-2450 double beam spectrophotometer in ‘spectrum’ mode using disposable, 10 mm path length, PMMA cuvettes. The instrumental baseline was recorded using two cuvettes with ultrapure water. For all subsequent analyses, a cuvette with ultrapure water was placed in the reference position of the spectrophotometer.

2.2.4.1. Determination of the TBO applicable concentration range.

To establish the applicable TBO concentration range for the experiments, absorbance spectra were recorded for TBO concentrations up to 73 \( \mu \)M. All spectra show a maximum absorbance at 632 nm, which corresponds to TBO in its monomeric form [28]. The molar extinction coefficient, derived from the slope of a plot of absorbance \( (A) \) versus TBO concentration, was 40,500 \( L \cdot mol^{-1} \cdot cm^{-1} \), which is in agreement with results from recent studies [29]. Up to a concentration of 40 \( \mu \)M, the TBO concentration dependence of the measured absorbance adheres to Lambert-Beer’s law, while at higher concentrations deviations are seen due to TBO self-aggregation into dimers (spectral data and Lambert-Beer-plot given in SI).

2.2.4.2. Determination of alginate-TBO binding stoichiometry

For the study of the alginate-TBO binding stoichiometry various compositions were made by a titration approach, also known as the method of continuous variation. A 50 mL solution of TBO at a fixed and defined concentration was prepared in buffer solution and an absorbance spectrum was recorded. Next, the alginate stock solution was added...
stepwise using calibrated micropipettes. After each addition, the newly established composition was mixed and equilibrated for 2 min prior to taking an aliquot on which the absorption spectrum was recorded. The composition of solutions was expressed as P/D, the dimensionless ratio of alginate (polymer) concentration P over the TBO concentration D (both expressed in moles/liter). The quantity P/D is used throughout the paper. TBO binds cooperatively to alginate and forms stacked assemblies leading to the appearance of a new metachromatic peak which is blue-shifted (hypsochromic shift). The stoichiometry was determined by plotting the ratio of the absorbance of this new metachromatic peak $A_m$ over the monomeric TBO peak $A_M$. All absorbance spectra were corrected for changes in reagent concentration caused by volume increase due to the addition of reagents. To investigate the influence of addition sequence on complexation, a reverse titration was done; i.e. TBO was added to a solution, in a stepwise fashion, with a fixed alginate concentration.

2.2.4.3. Displacement studies on alginate-TBO complexes with different composition. Alginate-TBO complexes with a predefined, variable composition P/D, were prepared (again, in a 1 mM phosphate buffer, pH 7) and once equilibrated, submitted to a similar titration approach in which cationic surfactant (TEGO), or anionic surfactants (SDS and SLES), were added to compete with either alginate or TBO. The same analytical methodology as described before was used; i.e. spectral data were recorded of the initial alginate-TBO complex and after each addition of the competing species. Data are presented as the ratio of metachromatic over monomeric peak height $A_m/A_M$ versus ratio of displacing ion over either P or D. Exact complex composition and further experimental details are given in the results section.

3. Results and discussion

3.1. Determination of the ‘apparent’ pK$_a$ of alginate under experimental conditions

Haug [30] reported the pK$_a$ values of guluronic and mannuronic acid of 3.65 and 3.38 respectively. In this publication Haug also notes that the pK$_a$ of the polymer alginate species is only slightly different from the pK$_a$ of the monomeric units. This statement has been adopted in later publications [15], without mentioning that Haug’s analyses were performed in solutions of high ionic strength (aqueous sodium chloride solutions, [NaCl] $\geq$ 0.1 M). In this study we envisage to work under conditions of controlled pH and much lower ionic strength. Weak polyelectrolytes, such as alginate, can be characterized by an ‘apparent’ pK$_a$ which is, depending on ionic strength, shifted to higher values with respect to the pK$_a$ of the single monomeric groups [31]. This pK$_a$ shift can be explained by the resistance of the carboxylic acid groups to deprotonate. When the carboxylic acid groups are close to another and the Debye length exceeds the distance between them there is an additional electrostatic penalty for dissociation. In a previous study, involving the same dye TBO [22], we observed that a constant pH can be maintained, whilst giving little to no interference with complexation, using a phosphate buffer at $C_{buffer} = 1$ mM and pH = 7. To verify whether this same buffer system was applicable, we determined the apparent pK$_a$ of alginate in this buffer. Fig. 1 shows the results of the apparent pK$_a$ determination using potentiometric titration and surface tension measurements.

From the two analyses reported in Fig. 1 it follows that the apparent pK$_a$ of alginate, under our experimental conditions, is 4.6 (+ 0.1), which is more than one pH-unit higher than the weighted average of the monomeric groups and in line with values reported by others [31]. The choice of a buffer at pH = 7, being 2.4 pH-units above the pK$_a$ of alginate, warrants that alginate will be fully deprotonated (> 99%) and all anionic binding sites available.

3.2. Determination of alginate-TBO stoichiometry

To use alginate as a template for binding TBO to perform displacement studies later on, the binding stoichiometry was investigated. In previous work we have demonstrated that step-wise construction of polymer-dye complexes, whilst monitoring the dye’s metachromasy, is a facile method to determine stoichiometry [32]. In Fig. 2 absorbance spectra are plotted that were obtained during stepwise construction of the alginate-TBO complex. Fig. 2 shows the development of a metachromatic peak resulting from the stacked organization of TBO whilst bound to adjacent binding sites on alginate. The stoichiometry of complexation is commonly determined by plotting the ratio of the metachromatic peak height $A_m$ over the monomeric peak height $A_M$ [10], as shown in Fig. 3. From the maximum in the curve ‘P/D high-to-low’, and the apparent discontinuity in the curve ‘P/D low-to-high’ in Fig. 3, it appears that alginate and TBO form a 1:1 stoichiometric complex, i.e. each anionic site on alginate (electrostatically) binds a single TBO ion. Furthermore it can be seen that at P/D $\leq$ 1, the complexation appears independent of the path followed as both experiments give similar results. However, at P/D $>$ 1 the two experiments follow a distinctly different path which can be explained as follows.

The experiment ‘P/D low to high’, essentially starts with only TBO (at [TBO] = 40 μM), to which stepwise alginate is added. Each newly added alginate molecule will be surrounded by an excess of TBO ions, leading to the immediate filling of the available binding sites on that molecule. The increase of the ratio $A_m/A_M$ continues until an equimolar concentration of alginate monomeric groups have been added (P/D = 1). Further addition of alginate induces an excess of alginate molecules, which may allow TBO redistribution to more favorable

![Fig. 1. Two ways of analysing the ‘apparent’ pK$_a$. Left panel: potentiometric titration, volume of added HNO$_3$ versus measured pH of alginate. Right panel: air-aqueous salt solution surface tension as a function of pH. In both cases pH was changed from high to low using 0.1 and 0.01 M HNO$_3$. Solution conditions: phosphate buffer 1 mM, pH = 7, $C_{alginate} = 0.64$ mM and 8.0 mM for resp. titration and surface tension measurement.](image-url)
locations as is suggested by the continued increase of Am/AM. Alginate is said to comprise three distinct monomeric distributions; G-blocks, M-blocks and alternating MG-blocks [15]. The inter-charge distance in these three different blocks may vary, causing TBO to favor one type over the other two, which would explain redistribution once more of these more favorable binding sites are offered. TBO does not fully redistribute into a monomeric distribution over alginate when a larger excess of alginate molecules are added, as Am/AM only slowly increases up to P/D \approx 10. This clearly demonstrates the cooperativity in TBO binding to alginate. Finally, when P/D > 10, the large excess of alginate binding sites seems to cause a further redistribution of TBO, possibly into smaller stacked organizations along the newly added alginate binding sites leading to a slow decrease in Am/AM. The experiment ‘P/D high to low’ starts with alginate only ([alginate] = 40 μM) to which TBO is added in a step-wise fashion. As can be seen from the initial low values of Am/AM, TBO at first seems to randomly distribute itself over the excess of alginate binding sites present. At P/D ≤ 10, and judged from the increase of Am/AM, cooperative binding of added TBO takes place until P/D = 1. At P/D < 1, newly added TBO added fails to find a binding site, and will remain in solution in its monomeric form, thus leading to an increase in Am at a near constant Am, as a result of which Am/AM decreases. Thus, next to information on the stoichiometry, Fig. 3 contains interesting information on the cooperativity of binding of TBO to alginate.

3.3. Displacement of TBO from alginate-TBO complexes by the cationic surfactant TEGO

When adding the cationic surfactant TEGO to a pre-formed alginate-TBO complex, it will compete with TBO for a binding site on the negatively charged alginate chain. Turbidimetric titration of alginate with TEGO shows that the binding stoichiometry for TEGO on alginate is 0.95 (results in SI). To understand the influence of alginate-TBO complex composition on displacement, experiments were performed in which the fraction of binding sites occupied by TBO (Fb) was varied by addition of an increasing concentration of alginate to a fixed TBO concentration D = 40 μM. The fraction of binding sites Fb is defined as:

$$ F_b = \frac{D}{P} $$

Next, these pre-formed complexes were submitted to displacement studies in which TEGO was added in a step-wise fashion. As can be seen from the decrease of Am/AM with increasing S/P as shown in Fig. 4, TEGO displaces TBO from its bound state to alginate. The results obtained at the five different TBO-loadings (P/D or Fb) condense into a common point, estimated to be at S/P \approx 0.91, where it is considered that TBO is fully displaced from alginate. This is in agreement with the TEGO-alginate stoichiometry determined by
turbidimetric titration, where $S/P = 0.95$ (SI). Comparing the curves obtained at different dye loadings reveals that the displacement pathway differs depending on the dye loading. At the lowest P/Ds ($1.01 < P/D < 1.26$), which is near stoichiometric complex conditions, TEGO immediately competes with bound TBO for a binding site and displaces TBO, which results in an instant, gradual decrease of $A_m/A_M$. Once fully displaced $A_m/A_M$ is constant. For the experiments with the lower dye loadings ($P/D > 1.26$) $A_m/A_M$ remains constant initially, which demonstrates that TEGO first (primarily) binds to free binding sites on alginate. Once these binding sites are full, TEGO starts competing with, and displacing, TBO, resulting in a sharp decrease in $A_m/A_M$ until a minimum is reached where again all TBO is assumed to be displaced. This explanation is further corroborated by the fact that the $S/P$ value at the onset of the decrease of $A_m/A_M$ scales with the P/D of the initial alginate-TBO complex. TEGO’s instant and stoichiometric displacement of TBO, from its stacked conformation, suggests that TEGO also binds cooperatively. This cooperativity in binding for TEGO results from the fact that, next to an electrostatic interaction, the spacing between alginate’s charged groups seems favorable to allow TEGO’s alkyl chains, on neighboring molecules, to align for hydrophobic or van der Waals interaction.

3.4. Displacement of TBO from alginate-TBO complexes by the anionic surfactant SDS

The displacement of TBO from its bound state with a polyanion by oppositely charged surfactants, also termed ‘reversal of metachromasy’ was previously reported in literature [10,18]. The fact that anionic surfactants, like SDS, are able to displace TBO from its binding with a polyanion, is accomplished by competing with alginate as a binding motive [10,18]. Upon interaction with TBO, depending on SDS concentration S, SDS can form ion-pairs or self-organize into premicellar or micellar structures [33,34]. Interestingly, these premicellar structures also lead to TBO stacking and the appearance of a metachromatic peak. Fig. 5 shows the spectral data obtained during the displacement of TBO from a preformed alginate-TBO complex (at $P/D = 1$, $P = D = 40 \mu M$) by step-wise addition of SDS.

Fig. 5 (left panel) shows that the metachromatic peak, arising from the interaction of TBO with SDS, $A_{m,SDS}$ is shifted further down the visible spectrum, as a clearly identifiable, separate peak. When the peak height data from this figure are plotted as a function of S/D, as shown in Fig. 5 (right panel), all four curves show a distinct change in trend at a common point, at $S/D = 7$. At this point $A_M$ and $A_f$ show a maximum value, whereas $A_{m,alg}$ approaches a minimum value and conversely $A_{m,SDS}$ a maximum value. From these observations it is assumed that at $S/D = 7$, SDS has displaced TBO from its binding with alginate.

Analogous to the experiments in section 3.3, the influence of alginate-TBO complex composition ($D = 40 \mu M$, $P =$ variable) on displacement is investigated. Likewise, preformed alginate-TBO complexes with different P/D, or dye loading $F_b$, are submitted to stepwise addition of SDS. The results of these experiments are shown in Fig. 6.

Similar to displacement by TEGO, when SDS addition is expressed as S/D, all curves in Fig. 6 converge into a common minimum, indicating full displacement of TBO. For all complex compositions full displacement is realized at $S/D = 7$. A more accurate determination of the S/D for displacement is derived by plotting the S/P for the minimum in $A_m/A_M$, denoted as (S/P)*, versus P/D, as shown in Fig. 6 right panel. Applying curve fitting to these data gives:

$$\left( \frac{S}{P} \right)^* = \frac{a_0}{\frac{P}{S}^{a_1}}.$$

with $a_0 = 6.962$ and $a_1 = -0.973$. Assuming $a_1$ to be minus unity, which seems a reasonable assumption, and multiplying both sides of the equation with $P$, turns Eq. (2) into:

$$\frac{S}{D} \approx 7$$

In previous work, studying the interaction and organization of TBO with SDS and SLES surfactants [22], we established that depending on the SDS to TBO ratio, S/D, four different organizations of TBO-SDS can be distinguished: I: Ion-pairs, II: Premicellar aggregates, III: Dimeric distribution over (pre-)micelles and IV: Monomeric distribution over micelles. The estimated S/D ranges for the different TBO-SDS organizations are given in Table 1. The S/D-ratio at which SDS manages to displace TBO from its bound state to alginate lies within the region where TBO and SDS organize in a premicellar organization. The fact that this premicellar state is able to remove TBO from its stacked arrangement on alginate is an indication that the premicellar state is energetically more favorable. Also in the experimental results shown in Fig. 6 (left panel) it can be seen that there is an effect of TBO loading onto alginate. At P/D < 0.81 an initial rise in the ratio $A_m/A_M$ can be seen prior to a decrease which indicates the transfer of TBO from alginate to SDS pre-micelles. The cause of this initial rise is the presence of free TBO which already form ion-pairs in which $A_m$ is lower due to a lower molar extinction coefficient as a result of the presence of larger aggregate ion-pairs. As $A_m$ will be far less affected by this, the subsequent increase of $A_m/A_M$ is caused solely by the decrease in $A_M$. Surprisingly, already from P/D ≥ 0.81 (or $F_b = 1.23$), where free TBO is still present, the curves all take up the same shape. Similar to the results for TEGO, the onset and steepness of the decrease in $A_m/A_M$ again seem to scale with the P/D. Exceptions, especially the experiment at P/D = 9.33, are caused by slight variations in starting conditions.
leading to a different $A_m/A_M$ at start which is maintained prior to the decrease in ratio. Summarizing, the premicellar arrangement of TBO with SDS is the main cause for TBO’s displacement from its bound state on alginate.

### 3.5. Displacement of TBO from alginate-TBO complexes by SLES surfactants with varying EO-block size

To investigate the influence of the EO-block size in SLES on their ability to displace TBO from alginate-TBO complexes, a similar approach is followed as given in section 3.4. Given the consistency of data obtained at a single P/D, experiments are only performed at P/D $\approx 1$ ($\pm 0.02$ except SDS at P/D $= 1.077$).

Fig. 7 summarizes the results expressed as the S/D ratio required to fully displace TBO from its complexed state with alginate (given as ($S/D$)$^*$) versus the size of the EO-block. Fig. 7 shows that the presence of a small EO-block is favorable for displacement as for (EO)$_2$ and (EO)$_4$ less surfactant is needed than for SDS, without an EO-block. Larger EO-blocks on the other hand require a higher concentration than SDS, making the ranking (EO)$_2 >$ (EO)$_4 >$ SDS $>$ (EO)$_{12} >$ (EO)$_{30}$. This observed order in efficiency of displacement is identical to the extent of premicellar aggregation for the same surfactant species with TBO, obtained by a different experimental setup in a previous study [22]. Comparison of the S/D values for displacement, shown in Fig. 7, corresponded well with the estimated S/D-range for premicellar aggregation shown in Table 1 (taken from [22]). The only exception is the SLES with 30 EO-groups, which requires S/D $= 17$, which falls outside the estimated premicellar range (S/D $= 5-11$). In this last case the premicellar arrangement is far less pronounced and thus more difficult to estimate. It may also be that, with this surfactant, TBO favors a dimeric organization.

As Fig. 7 shows, the displacement of TBO by SLES, driven by the significance and composition of their premicellar aggregates, is governed by the size of the EO-block and an optimum is observed for the SLES with an EO-block size of 2 units. The existence of this optimum may be explained as follows. When comparing the critical micelle concentration (CMC) of SDS and SLES with (EO)$_1$, the addition of the single EO-groups leads to a reduction of almost a factor ten (resp. 8.4 mM versus 0.89 mM), which is ascribed to intermolecular ion-dipole interactions between the sulfate ions and the $O \rightarrow$ CH2 dipole of the EO groups in SLES micelles [35]. Aoudia et al. [35] further show that the addition of two more EO-groups does not significantly alter the CMC and thus the intermolecular interaction. The decrease of $(S/D)^*$ in Fig. 7 from SDS to (EO)$_2$ and (EO)$_4$ may thus be explained by the fact that these SLES assemble at lower concentration thereby providing a suitable binding template at lower S/D. Once the EO-block size exceeds a certain size, flexing and bending may occur. Binding to a stacked array of TBO will decrease the possibility of this movement in the EO-block, which is entropically unfavorable. Therefore higher surfactant concentrations, and thereby also higher S/D, are needed to force the SLES (EO)$_{12}$ and (EO)$_{30}$ to assemble into a suitable binding template.

From the above results it is clear that, although the most likely mechanism for reversal of metachromasy of TBO is similar for different SLES surfactants, the S/D region in which premicellar aggregation occurs for a specific surfactant differs making this not a generic but a ‘surfactant structure’ dependent effect.

### 4. Conclusions

The ‘reversal of metachromasy’ or displacement of TBO from alginate-TBO complexes by surfactants was studied by a titration approach using visible light spectrophotometric methods. The suitability of the alginate-TBO complex as a model was firstly investigated by determining the ‘apparent’ $pK_a$ of alginate under the chosen experimental conditions as in Fig. 1.
conditions of controlled pH and ionic strength (phosphate buffer C_{buffer} = 1 mM, pH = 7) and was found to be 4.6, while its individual carboxyl groups have pK_{a} values of 3.38 and 3.65. Next, by applying a step-wise construction of the complex, the alginate-TBO stoichiometry was determined and found to be P/D = 1. It was shown that TBO binds cooperatively, caused by dye-stacking, to alginate, leading to the appearance of a metachromatic shift in the dye’s absorption spectrum. Having established the spectral properties of alginate-TBO complexes, these were used to study the ‘reversal of metachromasy’ or dye displacement by the addition of both cationic and anionic surfactants. For the cationic surfactant TEGO®trant A100 (DDMICl), which competes directly with TBO for a binding site on alginate, the displacement is stoichiometric with respect to available binding sites on alginate. The pathway of displacement was found to be depending on dye loading (P/D) and revealed that TEGO® first binds to empty bindings sites prior to its arrangement in a similar stacked organization leading to cooperativity in binding. From this observation, it is envisaged that intermediate alginate-dye-surfactant complexes consist of blocks in which either dye or surfactant are organized in stacked formation. For anionic SLES surfactants, it was found that displacement is governed by their ease of arranging into premicellar organization, which depends on surfactant structure and composition. Further spectroscopic studies may shed light on the specific interactions involved in the formation of these premicellar structures.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfa.2017.06.027.

**References**