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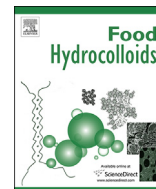
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Determination of the ‘apparent pK_a ’ of selected food hydrocolloids using ortho-toluidine blue

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ABSTRACT

Charged food hydrocolloids provide structure and texture in manufactured foods by their organization and electrostatic interactions with other species and small ions. These electrostatic interactions depend on their actual charge density which cannot be predicted from pK_a values of weakly charged monomers. In practice, this is circumvented by the use of an ‘apparent pK_a ’ ($pK_{a(\text{app})}$) which depends on solution ionic strength and type of counter-ions. $pK_{a(\text{app})}$ values for food hydrocolloids are poorly documented, especially in low ionic strength solvents. Titration of a metachromatic alginate-ortho-toluidine blue complex revealed a sigmoidal, pH-dependent, dye metachromasy response with its centre located at $\text{pH} = pK_{a(\text{app})}$. To test the suitability of this approach for the determination of $pK_{a(\text{app})}$, five food hydrocolloids and a synthetic polyanion are analysed and the results are compared to classical methods such as potentiometric titration. Metachromasy is shown to occur when the polymer inter-charge distance is 4.5 Å; the complex stoichiometry depending on inter-charge distance. $pK_{a(\text{app})}$ of stoichiometric complexes at low ionic strength (1 mM phosphate buffer) were successfully determined (alginate 4.5, sodium carboxymethylcellulose 4.6, κ -carrageenan 1.2, polyphosphate 2.1 and polyacrylic acid 5.8) and are comparable to values obtained by potentiometric titration. Further, it is shown that $pK_{a(\text{app})}$ determination by metachromatic dye titration is feasible for stoichiometric polymer-dye complexes at low concentrations (20–200 μM) in low ionic strength (<10 mM) aqueous solutions. Unlike classical methods, the metachromatic dye titration approach is suitable for low polymer concentrations in aqueous solutions at low ionic strength.

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1. Introduction

In formulated food products, hydrocolloids are indispensable components that impart key functionalities like thickening, gelling, stabilization and emulsification. These functionalities contribute to society's increasing demands with respect to desired properties of food such as ease of preparation, taste and texture (Vilgis, 2012), shelf-life (Gomez, Ronda, Caballero, Blanco, & Rosell, 2007), satiety (Fizman & Varela, 2013), health benefits (Gidley, 2013) and fat-replacement (Sudha, Srivastava, Vetrimani, & Leelavathi, 2007). Hydrocolloids are hydrophilic polymeric materials mainly consisting of (modified) polysaccharides or proteins from botanical, algal,

bacterial, or animal origin (Nishinari & Doi, 2012). In the case of polysaccharides, the hydrophilic nature is derived from the abundance of hydroxyl-groups which favourably interact with water, leading to, at least partial, solubility (Phillips & Williams, 2009). The richest functionality of polysaccharides is commonly found in species that also contain charged or chargeable groups like carboxylate (e.g. alginate, pectin, CMC, gellan) or sulphate (e.g. carrageenan) groups. Next to improved hydration and water solubility, the presence of charged groups also induces electrostatic interactions with oppositely charged species such as small ions, proteins, particles or other polyions, allowing the formation of structure and the stabilization of interfaces (Dickinson, 2003). Besides stabilization also destabilization may be observed. When the charged state of the polysaccharide changes, its effectively repulsive interaction with for instance proteins can induce depletion-driven flocculation or phase separation (De Kruif & Tuinier, 2001).

Charged polysaccharides are polyelectrolytes, which are known

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to be able to strongly adsorb, or complex, mainly through electrostatic interactions (Crouzier, Boudou, & Picart, 2010). Due to the proximity of neighbouring charged groups on polyelectrolyte chains, especially in case those are weakly charged groups for instance carboxylic acid groups, their actual charge density depends on solution conditions like pH, ionic strength and type of counter ions. Since charge density governs key factors for hydrocolloid functionality, such as solvability, structure and the ability to engage in interactions, this is a crucial parameter for its practical performance in foods (De Jong & van de Velde, 2007). Shiratori and Rubner (2000) provide a clear example of the effect of charge density on interactions and resulting structure, describing the pH-dependent thickness variation of sequentially adsorbed layers of weak polyelectrolytes.

As described by Katchalsky, Shavit, and Eisenberg (1954) the charge density of weak polyelectrolytes does not follow the classical Henderson & Hasselbalch equation as there is an additional thermodynamic penalty for removing a proton from an acidic group adjacent to an already deprotonated acidic group. This leads to the addition of a complex term to the Henderson-Hasselbalch equation which accounts for ionic strength, counter ion type and intrinsic polymer properties like chain conformation and flexibility. Strong polyelectrolytes are less affected by pH but can also exhibit counter ion condensation, as described by Manning (1978), causing them not to attain their full charge density. There are currently no mathematical solutions relating weak polyelectrolyte charge density to solution pH. Instead a pragmatic approach is chosen where an 'apparent pK_a ' is defined which is substituted in the Henderson-Hasselbalch equation (Porasso, Benegas, van den Hoop, & Paoletti, 2000; Paoletti, Gilli, Navarini, & Crescenzi, 1997). 'Apparent pK_a ' ($pK_{a(\text{app})}$) values, unlike pK_a values of monoprotic acids, are not constant but dependent on the ionic strength, polymer concentration and type of counter ions in the solution.

Surprisingly, $pK_{a(\text{app})}$ values are poorly documented. As a consequence, many studies base their interpretation of food hydrocolloid behaviour on 'monomeric' pK_a values. The monomeric pK_a , i.e. the pK_a of the monomer unit, however, is generally determined at high ionic strength where the polyelectrolyte-effect is annulled by the shielding of charges provided by the added electrolytes (Haug, 1964). When this monomeric pK_a value is applied to predict the behaviour of polyelectrolytes at low ionic strength, substantial deviations can be expected (Cooper et al., 2006). There are abundant methods to determine or predict pK_a values of mono- or diprotic acids and bases (Albert, 2012; Perrin, Dempsey, & Serjeant, 1981), however, most of these methods are, as such, not readily or easily applicable to polyelectrolytes. Some of the difficulties encountered in classical methods, for instance potentiometric acid/base titration, are that weak polyelectrolytes require a certain minimum concentration to be analysed accurately and the addition of acid or base to fully dissolve. At higher polymer concentrations, the addition of ions from the titrant is significant leading to a discontinuous solution ionic strength, which in turn causes slight shifts in $pK_{a(\text{app})}$. The presence of other acids or bases causes the titration curves to become more complex and sometimes difficult to interpret. Table 1 shows a selection of the documented experimental data for $pK_{a(\text{app})}$ values, under controlled solution conditions of, preferably, low ionic strength, relevant for the experimental work envisaged here.

Table 1 shows that true $pK_{a(\text{app})}$ values, in low ionic strength media, are scarce and that there is little experimental data available in recent publications. The above listing should be seen as an example of data published, rather than being a complete list.

In previous work (Vleugels, Ricois, Voets, & Tuinier, 2017) we reported on 'reversal of metachromasy' caused by the displacement of a metachromatic dye from its complexed state by surfactants. For

this purpose, we studied displacement of the planar dye ortho-toluidine blue (TBO) from its metachromatic, complexed state with alginate, by the addition of surfactant ions in solutions of low ionic strength. This displacement can be quantified by monitoring discrete changes in the dye's visible light spectrum i.e. the gradual disappearance of the metachromatic peak. In continuation of this work, we used protons and hydroxides as displacing ions and found that the data (spectrophotometric peak height ratios versus pH) yields a sigmoidal response with the centre located at a pH similar to the $pK_{a(\text{app})}$ of alginate found by potentiometric and liquid-to-air surface tension titration. This observation motivated us to further investigate the possibility of using metachromatic dye titration as a means of determining $pK_{a(\text{app})}$ of polyelectrolytes at low concentrations in dilute aqueous buffer solutions. To make a possible new method widely available, we continue to use UV-VIS spectrophotometry which is considered a routine method in both research and industrial laboratories. For this purpose, $pK_{a(\text{app})}$ values of sodium CMC, gellan, κ -carrageenan and pectin, and the synthetic polymers poly(acrylic acid) and poly(phosphate), are determined in aqueous buffer solutions and compared to values obtained by potentiometric and liquid-air surface tension titration methods. Finally, the influence of ionic strength and initial polyelectrolyte-TBO complex composition on the $pK_{a(\text{app})}$ are investigated.

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). Low acyl gellan gum, Kelcogel F, was a gift from CP Kelco. κ -carrageenan (Acros cat. nr. # 43159), carboxymethyl cellulose sodium salt (NaCMC) (molar mass \approx 250 KDa, DS = 1.2) (Acros cat. nr. # 33263) and pectin (degree of esterification 70%) (Acros cat. nr. # 41686) were purchased from Acros Organics. Poly(acrylic acid) sodium salt (NaPAA), Sokalan PA110S (molar mass \approx 250 KDa), was obtained from BASF. Polyphosphate, Calgon 322 new, was a gift from BK Guilini (Germany). Nitric acid 0.1N and potassium hydroxide 0.1N standardized solutions were purchased from Alfa Aesar, diluted ten times to reach 0.01N and standardized. Poly-diallyl-dimethyl ammonium hydrochloride (PDADMAC) 1.0 mM standardized titrant solution was purchased from BTG (Switzerland). 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 TBO, the food hydrocolloids and the polymer studied.

2.2. Methods

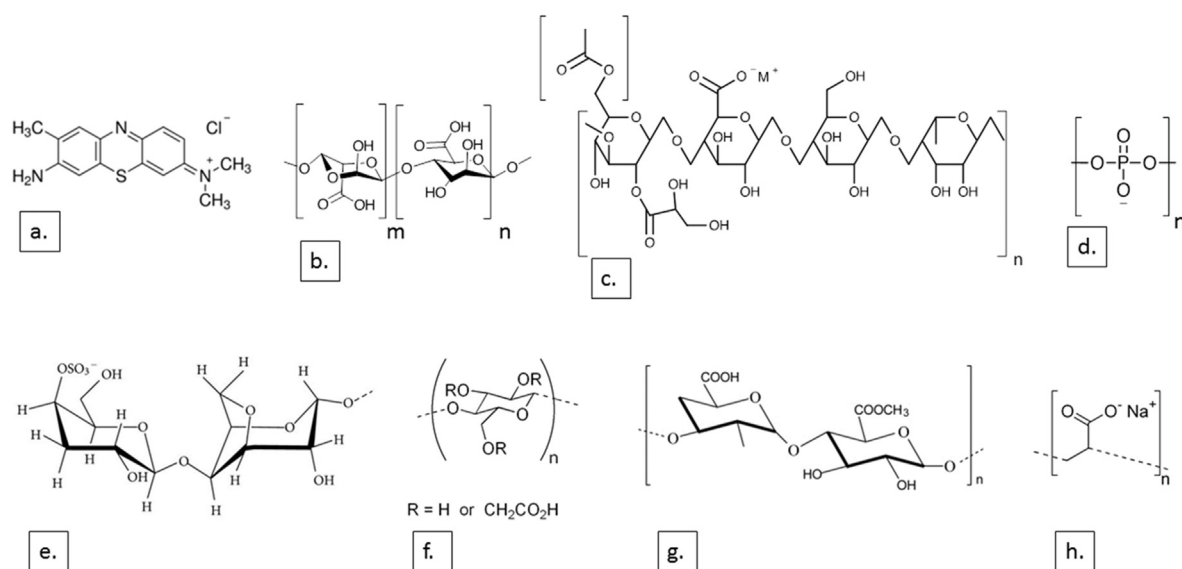
2.2.1. Solution preparation, definition and determination of 'functional' polyelectrolyte concentration (P)

The food hydrocolloids investigated in this study are not pure compounds and NaPAA is supplied as an aqueous solution (approx. 30 wt%). Therefore, at first, 10 mM solutions of all species were prepared in a freshly made 1 mM phosphate buffer, pH = 7.0, assuming the compounds to be 100% pure. The solubility, in this medium, for gellan and κ -carrageenan, proved insufficient as judged by the presence of insoluble particles. The 10 mM pectin solution developed a precipitate after a week. Therefore, these solutions were re-made with an estimated concentration of 1.0 mM. After overnight storage of the clear solutions, a few mg of sodium

Table 1
Overview of reported experimental data of 'pK_a' values of selected polyelectrolytes.

Polyelectrolyte species	pK _a	Ionic strength	Source	Remark #
Alginate	3.5	High	Haug, 1964	Monomeric pK _a
	4.6	Low	Rey-Castro, Herrero, & De Vicente, 2004	
Gellan	3.1–3.4	High	Milas, Shi, & Rinaudo, 1990	Monomeric pK _a
	3.4	High	Haug, 1964	Monomeric pK _a
κ-carrageenan	—	—	—	No exp. data found
NaCMC	3.91	Unknown	Vink, 1986	DS = 1.07*
	4.17	Unknown	Vink, 1986	DS = 1.69*
Pectin	3.4	High	Katchalsky et al., 1954	Monomeric pK _a
Polyphosphate	—	—	—	No exp. data found
Polyacrylate	4.68	High	Burke & Barrett, 2003	1 M NaCl
	5.1–5.3	Medium	Dickhaus & Priefer, 2016	0.01M NaCl
	6.21	Low	Dickhaus & Priefer, 2016	milli-q-water
	6.45	Low	Petrov, Antipov, & Sukhorukov, 2003	milli-q-water
	6.79	Low	Burke & Barrett, 2003	milli-q-water

Where monomeric pK_a is indicated, the pK_a value was determined at high ionic strength to determine the pK_a value of the monomer unit in the polymer. DS is the degree of substitution in this case the average number of substituted or charged groups per monomer unit.



Scheme 1. Generic molecular structures of (a.) TBO, (b.) alginate, (c.) gellan, (d.) Polyphosphate, (e.) κ-carrageenan, (f.) NaCMC, (g.) pectin, (h.) NaPAA.

azide was added to suppress microbial growth. As the polymers used in this study were foreseen as polyelectrolyte templates for the electrostatic binding of TBO, their 'functional' concentration P was defined as the equivalent number density (expressed as mM) of ionized groups at pH = 7. For each polyelectrolyte solution the quantity P , was determined by streaming potential titration (Barron et al., 1994; Tan, Norde, & Koopal, 2011), employing a Mutek™ PCD-05 Particle Charge Detector fitted with a standardized 1.00 mM PDADMAC titrant solution. To ensure a constant pH, titration was performed in the same phosphate buffer solution ($C(\text{buffer}) = 1 \text{ mM}$, pH = 7) and consisted of triplicate analyses which were averaged to obtain the functional polyelectrolyte P (data shown in Supporting information, from here on referred to as SI).

2.2.2. Determination of pK_a (app) values by previously reported methods

2.2.2.1. Potentiometric titration. The determination of pK_a (app) by potentiometric titration was performed using a Metrohm 907 Titrand titrator equipped with a calibrated glass electrode (Metrohm Aquatrode Plus) and standardized solutions of respectively 0.01M potassium hydroxide (KOH) and 0.01M nitric acid (HNO₃)

solution. An aliquot of the polyelectrolyte solutions was weighed into the titration vessel, containing a measured amount of phosphate buffer ($C(\text{buffer}) = 1 \text{ mM}$, pH = 7), to reach an actual concentration of 0.4 mM. This solution was firstly titrated with KOH titrant solution to pH ≈ 9 (forward titration), immediately followed by a back-titration of the titration mixture using HNO₃ titrant solution. The pK_a (app) was determined in the following manner. The equivalence volume in the forward titration, stemming from residual acid and further neutralization of the phosphate buffer, was subtracted from the total volume required to reach pH ≈ 9 giving the excess of KOH added. Next, this excess was subtracted from the volumetric addition of HNO₃ in the back-titration. pK_a (app) was defined as the pH-value at 1.5 times the equivalent volume obtained from the 'volume-corrected' back-titration curve. Triplicate titrations were performed and the resulting pK_a values were averaged.

To investigate the influence of solution ionic strength, the same experimental approach was used in a titration medium in which part of the buffer solution was replaced by a sufficient volume of 0.1 M potassium chloride (KCl) solution (in 1 mM phosphate buffer, pH = 7.0) which was added to obtain concentrations of 1, 3, 5 and 10 mM added KCl.

2.2.2.2. Liquid-air surface tension titration. A novel titration method, recording surface-to-air tension as a function of pH, for the determination of apparent pK_a values was recently reported by Dickhaus and Priefer (2016). This methodology was applied, employing an Attension Sigma-70 tensiometer using the Wilhelmy-plate method. An amount of 40 g of polyelectrolyte stock solution ($C(\text{polymer}) \approx 10 \text{ mM}$) in phosphate buffer ($C(\text{buffer}) = 1 \text{ mM}$, $\text{pH} = 7.0$) 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 averaged. Next, the pH of the solution was stepwise lowered by volumetric addition of 0.1 M or 0.01 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. 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.

2.2.2.3. Electrophoretic mobility titration. Only for alginate, a single experiment series for apparent pK_a determination was performed using Malvern Zetasizer NANO ZS (Worcs, UK) capable of performing micro-electrophoresis analysis. A similar titration approach, as described in before, was performed. After each HNO_3 addition, the solution was allowed to equilibrate for 10 min, followed by analysis of pH. Subsequently, the ζ -potential of an aliquot was determined in triplicate. The ζ -potential values at each pH were averaged and plotted versus pH. Extra attention was given to the instrument's photon count rates during each measurement. The pK_a was determined as the inflection point in a sigmoidal fit performed on the ζ -potential values versus solution pH.

2.2.3. Determination of apparent pK_a of polyelectrolytes using polyelectrolyte-TBO-complex metachromasy

2.2.3.1. Determination of polyelectrolyte-TBO complex stoichiometry. Prior to determination of the polyelectrolyte apparent pK_a , the formation and stoichiometry of metachromatic TBO-complexes with all the studied polyelectrolytes were investigated in a buffered solution (phosphate buffer $C_{\text{buffer}} = 1 \text{ mM}$, $\text{pH} = 7.0$). The methodology for this determination has been described in a previous paper (Vleugels et al., 2017). In summary, different complex compositions were prepared in a titration approach, in which the polyelectrolyte of interest was step-wise added to a buffered TBO solution at $C_{\text{TBO}} = 40 \mu\text{M}$, and analysed for their visible absorbance spectrum (wavelength (λ) scan 800–350 nm), employing a Shimadzu UV-2450 UV-VIS double beam spectrophotometer. When metachromasy occurred, due to stacking of TBO onto the polyelectrolyte template, the absorbance spectrum of the polyelectrolyte-TBO complex displayed a new, distinct, absorbance peak from here on referred to as the metachromatic peak. From the absorbance spectrum, which was corrected for volumetric addition of reagents, the TBO monomer peak height (A_M) and the metachromatic peak height (A_m) were determined. Next, the ratio A_m/A_M was plotted versus the ratio P/D (in which P is the 'functional' polyelectrolyte concentration determined as described in 2.1.1 and D is the dye concentration) and overlaid with plots of the quantities A_m and A_M . The complex stoichiometry (P/D) was estimated from the most distinct change in trend in all three curves (an example is shown in the results section).

2.2.3.2. Determination of polyelectrolyte pK_a (app). Once the existence of a metachromatic complex between the studied polyelectrolyte and TBO, and its stoichiometry, was established, the displacement of TBO by other ions could be studied by applying a

titration approach in which the extent of metachromasy (indicated by the ratio A_m/A_M) was followed as a function of the added amount of displacing ion. This approach, successfully used for surfactants in our previous work (Vleugels et al., 2017), was applied to displace TBO by H_3O^+ and OH^- . In practice, a 50 ml portion of a stoichiometric complex of TBO and the polyelectrolyte of interest was prepared in buffer solution. To obtain comparable spectral data with sufficient sensitivity, the TBO concentration was fixed in all experiments at $C_{\text{TBO}} = 40 \mu\text{M}$ and the added polyelectrolyte concentration was adapted accordingly to meet the previously found stoichiometry. After the pH was registered, a visible absorbance spectrum (wavelength scan $800 \geq \lambda \geq 350 \text{ nm}$) was recorded on an aliquot of the solution. Next, HNO_3 solution ($C = 0.10$ or 0.01 N) was added, using calibrated micropipettes to lower the pH. The solution was allowed to equilibrate for 1 min, during which, in all experiments, a stable pH value was attained. The solution was sampled and the absorbance spectrum recorded. This was repeated until the absorbance spectrum showed negligible variation upon further lowering of pH. Additionally, and only for alginate, an experiment series was performed in which the final solution of the previously described approach was back-titrated to neutral pH using KOH. After correction of all spectral data for the volumetric addition of HNO_3 , the absorbance peak heights A_m and A_M were determined. The apparent pK_a was obtained from the inflection point (X_0) in a sigmoidal or Boltzmann-fit of A_m/A_M values plotted against solution pH.

2.2.3.3. Determination of the influence of ionic strength and polyelectrolyte-TBO complex stoichiometry. To investigate the influence of solution ionic strength on the apparent pK_a , potentiometric and TBO-polyelectrolyte complex titrations were performed, as described above, with the addition that prior to starting the titration, part of the buffer solution was replaced by equal, measured amounts of a 1.0 M potassium chloride (KCl) solution to obtain final solution concentrations of 1, 3, 5 or 10 mM KCl. The influence of polyelectrolyte-TBO complex stoichiometry was investigated by adjusting the amount of added polyelectrolyte to a fixed TBO concentration of $40 \mu\text{M}$. Further experimental details in this case are presented in the section Results and discussion.

3. Results and discussion

3.1. pK_a (app) of alginate

3.1.1. TBO metachromasy tested as a tool for pK_a (app) determination

In our previous work (Vleugels et al., 2017) we determined the apparent pK_a (pK_a (app)) of alginate to assess its suitability as a template for the binding of the cationic, metachromatic dye orthotoluidine blue (TBO). Potentiometric and surface tension titration of alginate in 1 mM phosphate buffer both yielded a value of pK_a (app) = 4.6. This value is higher than the monomeric pK_a values reported for D-guluronic and D-mannuronic acid which are respectively 3.65 and 3.38, as determined by Haug (1964) using a sodium chloride solution ($[\text{NaCl}] \geq 0.1 \text{ M}$) as solvent. The difference between monomeric pK_a and pK_a (app) is a direct consequence of the fact that the weakly carboxylic acid groups along the polyelectrolyte chain are in closer proximity than the Debye-Hückel length, (λ_D). As a result the dissociation of the acids groups requires an additional energetic (electrostatic) contribution.

The Debye length, λ_D , decreases with the square root of the inverse ionic strength (Debye & Hückel, 1923). This can be understood from screening of the charged carboxylate groups by additional counter ions in solution, thus effectively lowering the additional energetic penalty for dissociation which in turn leads to a lower effective pK_a . At high ionic strength, as was the case in

Haug's (1964) experiments at $[\text{NaCl}] \geq 0.1 \text{ M}$, the energetic penalty is negligible and the effective pK_a values are equal to those of the acid groups on the individual monomer units. When submitting a pre-formed charge-stoichiometric alginate-TBO complex to displace TBO with hydronium (H_3O^+) ions, we observed a decrease of metachromasy (shown in Fig. 1) caused by direct competition of H_3O^+ with TBO for a binding site on the alginate chain. Displacement of TBO from alginate inherently leads to a reduction of the number of TBO ions present in a stacked metachromatic conformation, thus lowering the metachromatic peak height. As the TBO molecules are released into the solvent as individual dye ions, conversely an increase in TBO's monomer peak height is observed. When H_3O^+ displaces TBO it also neutralizes the carboxylic acid group. To investigate the relationship between TBO displacement and pH, the extent of metachromasy expressed as the ratio between metachromatic and monomer TBO peak height (A_m/A_M), is plotted as a function of solution pH as shown in Fig. 1. TBO displacement is reversible (re-assembling of the metachromatic complex) as shown in Fig. 1, when the solution pH is raised again using hydroxide (OH^-) from potassium hydroxide.

The sigmoidal, or Boltzmann, curve fitting was performed by Originlab[®] Origin 6.1 software which uses the following equation [1] to determine X_0 or pK_a :

$$\left(\frac{A_m}{A_M}\right) = \frac{\left(\frac{A_m}{A_M}\right)_{\min} - \left(\frac{A_m}{A_M}\right)_{\max}}{1 + e^{\frac{\text{pH} - \text{pK}_a}{\Delta\text{pH}}}} + \left(\frac{A_m}{A_M}\right)_{\max} \quad (1)$$

in which $\left(\frac{A_m}{A_M}\right)_{\min}$ and $\left(\frac{A_m}{A_M}\right)_{\max}$ are the minimum and maximum observed ratios, resp. at low and high pH. The pK_a (app) values of 4.40 and 4.53 (respectively for high-to-low and low-to-high pH), as derived from the sigmoidal fit as shown in Fig. 1, are in good agreement with those obtained with potentiometric and surface tension titration (Vleugels et al., 2017). Next to that, they are close to a previously reported value of 4.6 (Rey-Castro et al., 2004).

The absolute A_m/A_M values in the reverse titration show that the metachromasy is not fully restored, which is most likely due to the fact that when fully neutralized alginate tends to aggregate and these aggregates may not fully re-dissolve during the experiment. However, the qualitative trend gives a very similar value for X_0 from

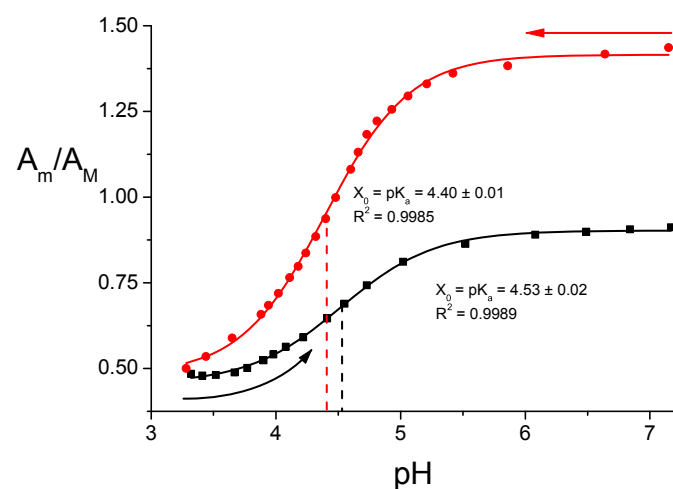


Fig. 1. Displacement of TBO from alginate-TBO stoichiometric complex by H_3O^+ (dots) and OH^- (squares) expressed by the ratio A_m (absorbance metachromatic TBO peak), and A_M (absorbance monomer TBO peak), versus solution pH (in phosphate buffer $c = 1 \text{ mM}$). The solid curve represents the fitted sigmoidal functions to both data sets and the centre of the sigmoidal (indicated by the dashed line), X_0 , represents pK_a (app).

which the pK_a (app) is determined.

This finding; e.g. this method of determining apparent pK_a (app), has, to the best of our knowledge, not previously been reported in literature. To test the validity of this approach for determining pK_a (app), we selected and investigated five additional polyelectrolytes commonly used in food (polyphosphate, κ -carrageenan, sodium carboxymethyl cellulose, pectin and gellan) and one synthetic, weak polyanion (sodium polyacrylate, NaPAA) and compared their pK_a (app) determined by TBO metachromasy and conventional methods (potentiometric and surface tension titrations). These results are shown in section 3.2.

3.1.2. Evaluation of micro-electrophoretic titration to determine apparent pK_a ; the potential risk of error

In recent literature the dissociation of polyelectrolytes, as such or as elements of an assembled structure, has been determined by measuring the zeta potential (ζ) as a function of pH (Burke & Barrett, 2003; Harnsilawat, Pongsawatmanit, & McClements, 2006; Kirby & Hasselbrink, 2004). The results of micro-electrophoretic titration of a 10 mM alginate solution in phosphate buffer yielding the ζ -potential are shown in Fig. 2.

The experimental results (squares) shown in Fig. 2 are compared to literature (triangles) data (Harnsilawat et al., 2006) and follow the same trend, which can be fitted to a sigmoidal function. Surprisingly, the pK_a (app) value derived from the sigmoidal fit suggests a value of pK_a (app) = 2.85 ± 0.06 , which is significantly lower than obtained by other methods (section 3.1.1). The explanation the authors offer for the observed trend is the continued protonation of carboxylic acid groups on alginate which is further motivated by quoting the monomeric pK_a of 3.5 (citing Draget et al., 2000, which in turn cite Haug, 1964). Following a further evaluation of our data and the observation that the alginate solution becomes slightly turbid when the pH is lowered from 5 to 4, we postulate an alternative explanation for the observed behaviour. To arrive at our insight it is useful to focus on the measured scattering count rates as a function of pH, see the insert in Fig. 2. At $\text{pH} > 5$, it is assumed that alginate is present as individual polymer chains, which scatter little light. The scattering count rates, however, show a distinct rise when the pH is lowered from 5 to 4. Once alginate starts aggregating and precipitating, the appearance of larger particles leads to

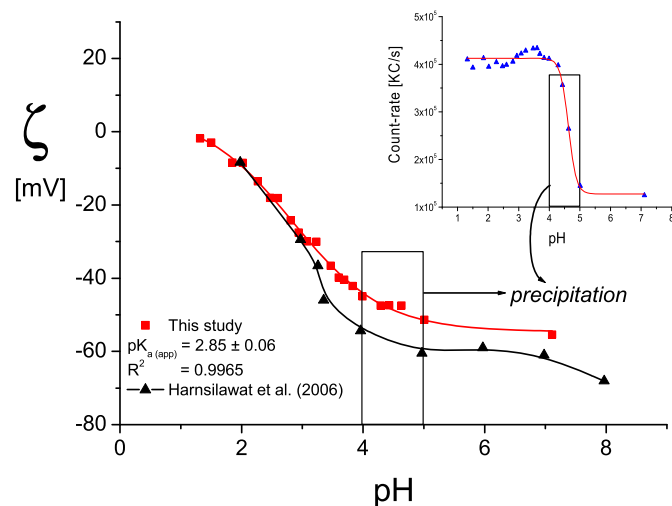


Fig. 2. ζ -potential titration of a 10 mM alginate in 1 mM phosphate buffer solution using HNO_3 (squares), compared to data adapted from Harnsilawat et al. (2006), (triangles). pK_a (app) derived as the X_0 value from sigmoidal fit. The hatched squares, both in figure and insert, indicate the visual appearance of precipitate. Insert: DLS scattering count-rate versus pH.

a marked increase in scattering as the scattered light intensity I (here expressed as count rate) scales as $I \propto d^6$ with d the particle diameter. Therefore, aggregated and/or precipitated particles, rather than alginate molecules, now dominate the determination of the ζ -potential. As these particles are prone to pick up indifferent ions, in this study e.g. phosphate from the buffer solution, the observed trend must be attributed to particles rather than to individual alginate molecules and therefore is considered erroneous. With this insight and the knowledge that the food hydrocolloids investigated here all have a tendency to aggregate when becoming uncharged, no further attempts were made to use electrophoretic mobility measurements to determine $pK_{a(\text{app})}$.

3.2. Apparent pK_a of the selected polyelectrolytes

3.2.1. Metachromasy and polyelectrolyte-TBO stoichiometry

To employ dye metachromasy as an indicator of the protonation state of polyelectrolytes in pH-titration, it is firstly required to establish whether metachromasy occurs for TBO and the polyelectrolyte of interest. Dye metachromasy can be the result of dye self-stacking at higher concentrations, but becomes far more pronounced in case there is a template present that binds the dye and guides its aggregation or stacking (Lison, 1935). Stacking is generally induced when the template allows the dye to bind at such short intermolecular distances that the individual dye ions can interact via hydrophobic or π - π interactions. Since both these interactions are relatively short-ranged, 4.4 Å at most (Avasthi, Shukla, Kant, & Ravikumar, 2014), if the inter-charge distance is larger, although the dye may still bind electrostatically, the TBOs will not display metachromasy. The inter-charge distance of a specific polyelectrolyte is both governed by molecular structure and conformation as well as by the chosen conditions such as polymer concentration, ionic strength and type of ions present. Therefore, experimental verification and establishment of metachromatic complex stoichiometry is required.

As shown in our previous work (Vleugels, Domańska, Voets, & Tuinier, 2017), metachromasy and the stoichiometry of a metachromatic polyelectrolyte-dye complex can be determined by a step-wise construction of the polyelectrolyte-dye complex in a titration-type approach whilst recording visible light absorption spectra. In this study the polyelectrolytes are stepwise added to a buffered TBO solution ($C_{\text{Dye}} = 40 \mu\text{M}$). The applicability of this approach for alginate has been reported in an earlier publication (Vleugels, Ricois, Voets & Tuinier, 2017). The reverse approach is also possible, adding TBO to a buffered polyelectrolyte solution, but as the dye concentration varies quite significantly also the spectral data show large differences in overall magnitude, which complicates the interpretation of data.

For each food hydrocolloid and polymer, TBO-polyelectrolyte metachromasy and complex stoichiometry are determined. Firstly, the determination of complex stoichiometry from spectral data is illustrated as an example for the NaCMC-TBO complex as presented in Fig. 3.

The spectra in Fig. 3 allow for determination of the TBO monomer peak height A_M , the metachromatic peak height A_m and the ratio A_m/A_M as a function of P/D . At $P/D = 0$, virtually all TBO is present in its monomer form, and therefore the monomer TBO spectrum is found. Upon increasing P/D , by addition of NaCMC, the monomer TBO peak, located at $\lambda = 631 \text{ nm}$, decreases and a metachromatic TBO peak, centred at $\lambda = 547 \text{ nm}$, appears and increases in magnitude. Comparing the trends of the data in all three curves allows identification of a common point (indicated in Fig. 3 by the vertical line at $P/D \approx 1.3$) at which all curves show, on average, the intercept fitted lines between two distinct trends and which is assumed to represent complex stoichiometry. This same method of

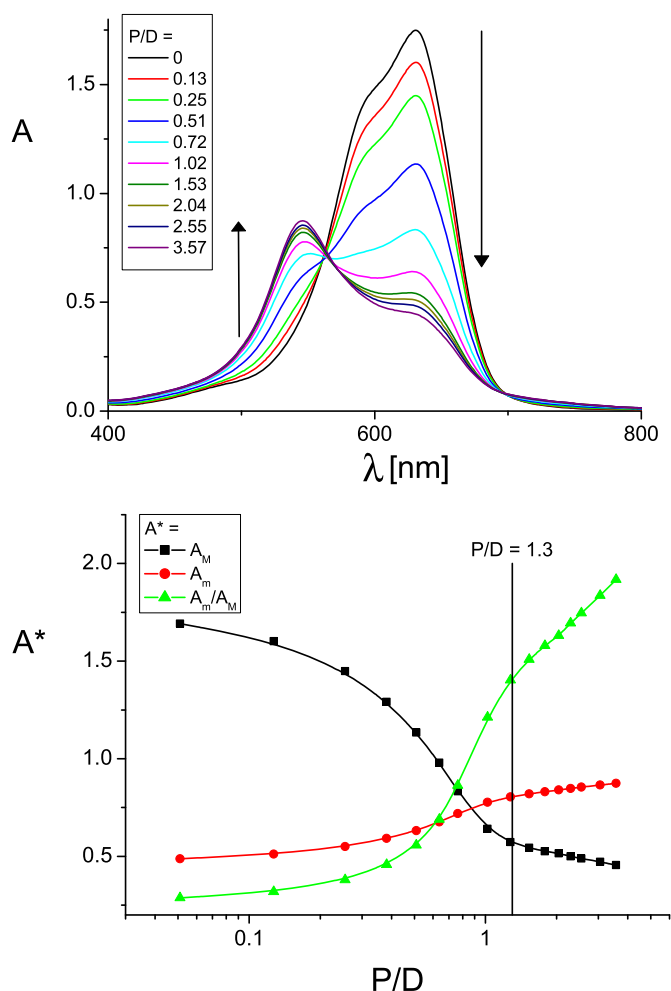


Fig. 3. Top: Absorption results for the determination of stoichiometry of the NaCMC-TBO complex. Spectral data as a function of complex composition; P/D . Arrows indicate the changes with increasing P/D . Bottom: Absorbance peak values for monomer dye peak height A_M (squares), metachromatic dye peak height A_m (closed circles) and ratio A_m/A_M (triangles) versus complex composition P/D . The vertical line at $P/D = 1.3$ does not represent experimental data but is added to guide the eye to the P/D value at which all three curves combined show an intercept between two distinctly different trends. Solution conditions: 1 mM phosphate buffer at $\text{pH} = 7.0$.

approximation was applied to all other experiments reported in Table 2.

The explanation for the trends observed in Fig. 3 is as follows. Upon addition of NaCMC to the TBO solution, TBO starts binding to the oppositely charged carboxylic acid groups on NaCMC. As the molar extinction coefficient of bound and stacked TBO is lower than that of the free dye, the monomer dye peak decreases with increasing P/D . Subsequently, given the fact that at $P/D < 1$ there is an excess of TBO relative to NaCMC's carboxylic acid groups, the metachromatic TBO peak height increases. This is most clearly visualized by plotting these opposing trends as the ratio A_m/A_M . The trend in A_m/A_M , after a gradual increase prior to $P/D \approx 0.6$, shows two distinct near-linear sections with different slopes. Complex stoichiometry is determined as the P/D at the intercept of two lines fitted through both sections. The fact that A_m/A_M does not level off but continues to rise when P/D is larger than stoichiometry, most likely resides in the fact that at higher P/D more and especially more favourable binding sites are present for TBO. This suggests TBO redistribution, which is possible as the number of polyelectrolyte binding sites now exceeds the amount of TBO ions

Table 2
Polyelectrolyte conformation and charge distance versus the appearance of metachromasy, TBO complex stoichiometry and the location of the metachromatic TBO peak.

Polyelectrolyte	Conformation	Inter-charge distance Å	Metachromasy	Stoichiometry P/D	λ_{meta} nm
Alginate	poly-M	10.4 ^a	Yes	1.0	547
	poly-M-G	10.3 ^a			
	poly-G	8.7 ^a			
Gellan	Solid state	5.6 ^b	No	Not detected	–
	Theoretical	17.3 ^f			
κ -carrageenan	Random coil	10 ^c	Yes	2.0	544
	Helix	4 ^c			
NaCMC	–	4.3 ^d	Yes	1.3	547
	–	5.2 ^e			
Pectin	–	6 ⁱ	No	Not detected	–
Polyphosphate	Random coil	2.6 ^g	Yes	3.8	547
NaPAA	Random coil	2.5 ^h	Yes	2.1	541

Legend to literature references in table 2: ^a Feng, Kopplin, Sato, Draget, and Vårnum (2017), ^b Morris, Nishinari, and Rinaudo (2012), ^c Vinceković, Katona, Bujan, and Sovilj (2011), ^d Zhivkov (2013), ^e Vink (1986), ^f Ralet, Crépeau, Buchholt, and Thibault (2003), ^g Cini and Ball (2014), ^h Maki, Yoshida, Nariai, and Mizuhata (2015), ⁱ Rinaudo and Milas (1974). – refers to values not determined/mentioned.

present. The NaCMC species tested here has an averaged degree of substitution of 1.2, however it is considered that the distribution of charged groups is random and locally there may be higher charge distances offering a more suitable template for TBO stacking. Following the same approach, the TBO complex stoichiometry was determined for the investigated polyelectrolytes, as shown in Table 2.

Table 2 reveals an interesting relation between TBO complex stoichiometry, metachromasy, and inter-charge distance of the investigated polyelectrolytes, which may be smaller, comparable or larger than the postulated ideal distance for TBO stacking (4.4 Å according to Avasthi et al., 2014). For inter-charge distances smaller than the postulated ideal distance for TBO, this leads to stoichiometry at $P/D \geq 2$. This is the case for NaPAA and polyphosphate with charge distances of 2.5–2.6 Å, and to a lesser extent for κ -carrageenan with an inter-charge distance in helical form of 4 Å and an observed stoichiometry at $P/D = 2$. In case the inter-charge distance is about equal to TBO's ideal stacking distance, stoichiometry is found at $P/D \approx 1$, which is the case for NaCMC and alginate. When the inter-charge distance exceeds TBO's ideal stacking distance, no metachromasy is observed and hence no stoichiometry can be determined from these results.

The explanation for this may reside in three types of structural TBO arrangements. In the first, when the inter-charge distance is smaller than the postulated ideal distance for TBO stacking (NaPAA, polyphosphate and to lesser extent κ -carrageenan). Here, as a result of this close proximity of charged groups, one TBO ion takes up similar space as multiple charged binding sites, which thus do not partake in further TBO-binding, leading subsequently to stoichiometry at $P/D \geq 2$. In the second structural TBO arrangement (observed for alginate and NaCMC), the inter-charge distance is approx. equal to TBO's ideal distance for stacking. In this case, stoichiometry close to unity is expected and actually alginate and NaCMC show stoichiometry respectively at $D/P = 1.0$ and 1.3. If one compares the reported inter-charge distances for alginate and NaCMC (Table 2), this inevitably implies that both polyelectrolytes assume a helical conformation to bring the inter-charge distance into the desired, ideal, distance of 4.5 Å. It may, however, be incorrect to assume TBO metachromasy can be used as an indicator for the formation of helical structures in food hydrocolloids as the energy gain from cooperative TBO stacking along the polymer chain may be the driving force for the observed helical conformations. In the third type of structural TBO arrangement (observed for pectin and gellan) the inter-charge distance exceeds the ideal stacking distance of 4.5 Å for TBO. In this case TBO may bind to the binding sites but fails to form stacked structures and hence no metachromasy is observed. Based on the observations here, under the

chosen experimental conditions, gellan and pectin also seem unable to accommodate for this by the formation of a helical conformation. The inter-charge distance quoted for gellan (Morris et al., 2012) refers to a solid state model, in which gellan may take up a helical conformation amounting to a charge distance of 5.6 Å. However, in solution individual gellan molecules can arrange in extended chains where the inter-charge distances is closer to the theoretical value listed in Table 2 which is based on the size of pyranose rings (4.35 Å, Rees & Wight, 1971). For the pectin species investigated here it is stated that the degree of esterification is 70%, which means that roughly only one out of three monomer units bears a charged or chargeable group. Similarly, in case pectin forms no helical structures, this leads to an actual inter-charge distance of 13.1 Å, which equals three times the size of the monomer unit being 4.35 Å.

When comparing the wavelength positions of the various metachromatic states in Table 2, little to no variation is seen, suggesting that TBO is present in similar sized stacks (D'Ilario & Martinelli, 2006) which are separated by non-stacked zones. Finally, as no metachromatic complex was formed between TBO and gellan and pectin, $pK_{a(\text{app})}$ values for these compounds cannot be determined by the TBO-metachromasy method presented here, conversely only potentiometric and surface tension titration data are given in the next section.

3.2.2. Determination of $pK_{a(\text{app})}$ by TBO metachromasy and comparison to potentiometric and surface tension titration

The applicability of the metachromatic dye titration method for the determination of $pK_{a(\text{app})}$ is investigated next, by performing regular pH titrations of pre-assembled, stoichiometric polyelectrolyte-TBO complexes while monitoring their spectral properties, i.e. the observed peak height ratio A_m/A_M . Prior to using TBO in pH-titrations a blank experiment involving only TBO ($[TBO] = 40 \mu\text{M}$, in 1 mM phosphate buffer, initial $\text{pH} = 7$) is performed. This experiment shows that TBO has the same spectral properties in the investigated range of $0.5 < \text{pH} < 10$; the data are presented in the SI. The resulting $pK_{a(\text{app})}$ values are compared to results obtained by potentiometric and surface tension titration. An overview of the results is shown in Table 3.

Table 3 demonstrates that the TBO metachromasy method is capable of determining $pK_{a(\text{app})}$ in all cases where metachromasy is observed. This also holds if the charged group has a relatively low pK_a value as is the case for a sulphuric or phosphoric acid-group. Compared to potentiometric and surface tension titration data, it follows the data from the different methods are overall in good agreement. For κ -carrageenan and polyphosphate the determination of $pK_{a(\text{app})}$ by potentiometric titration is not possible under the

Table 3
Polyelectrolyte charge–distance, type of charged group and $pK_{a(\text{app})}$ for the studied polyelectrolytes.

Polyelectrolyte	Type of charged group	Theoretical Inter-charge distance Å	$pK_{a(\text{app})}$ TBO metachromasy [#]	$pK_{a(\text{app})}$ potentiometry ⁺	$pK_{a(\text{app})}$ tensiometry [*]
K-Carrageenan	-SO ₃ H	4	1.2 ± 0.06	n.d.	n.d.
polyphosphate	-PO ₃ H	2.6	2.1 ± 0.10	n.d.	n.d.
NaPAA	-COOH	2.5	5.8 ± 0.10	5.8 ± 0.10	5.8 ± 0.07
NaCMC	-COOH	4.3	4.6 ± 0.03	4.8 ± 0.05	n.d.
Alginate	-COOH	8.7	4.5 ± 0.10	4.6 ± 0.05	4.6 ± 0.08
Pectin	-COOH	13.1	n.d.	4.6 ± 0.15	4.6 ± 0.03
Gellan	-COOH	17.2	n.d.	4.7 ± 0.07	n.d.

Polyelectrolyte concentration P: [#] at stoichiometry, variable 0.04–0.16 mM, ⁺0.4 mM and ^{*}10 mM.

chosen conditions; the HNO₃ titrant solution is too dilute to reach the required low pH-values, hence these values cannot be assessed. Using higher titrant concentration would lead to a higher ionic strength in the titration solution which is undesirable in our attempt to establish the $pK_{a(\text{app})}$ at a controlled, low ionic strength. For the surface tension titration we observe that in some cases (i.e. κ -carrageenan, polyphosphate, NaCMC and gellan) the initial surface tension results, at high pH, are already lowered (60–70 mN/m) when compared to ‘water-like’ (72 mN/m) values, reported by Dickhaus and Priefer (2016). This points at the presence of low molar mass surface active species present in the materials used. These low molar mass species may show dissimilar pH-dependent surface activity with the inherent risk of masking, or interfering with, the polyelectrolyte’s distribution over the liquid–air interface and bulk liquid phase and thus obscuring trends. Hence, only the results of the experiments that gave initial ‘water-like’ surface tension results and clear trends are listed in Table 3. Finally, compared to the other test methods, tensiometry titration is performed at 10 mM where P is respectively 25 and 250 times higher than for potentiometric or metachromatic titration.

When examining the observed $pK_{a(\text{app})}$ values for the investigated species, a previously unreported value for polyphosphate is found at 2.1 which is close to the monomeric pK_a value at 2.15. Given the short inter-charge distance and the finding that NaPAA, with a similarly low inter-charge distance has a substantial difference between $pK_{a(\text{app})}$ and monomeric pK_a (respectively 5.8 and 4.3), this is surprising. At present we cannot offer an explanation for this. The value obtained for κ -carrageenan, $pK_{a(\text{app})} = 1.2$, is in good agreement with the value for the sulphate group on poly(styrene sulfonate) reported by Dickhaus and Priefer (2016). For NaCMC, alginate, gellan and pectin the $pK_{a(\text{app})}$ values are roughly one pH unit higher as their monomeric pK_a values, which, given the low polymer concentration and low solution ionic strength, seems sensible.

3.3. The influence of ionic strength on $pK_{a(\text{app})}$ determination

To assess the influence of the solution ionic strength on $pK_{a(\text{app})}$, and its determination, a number of experiments are performed in which the ionic strength is increased by the addition of potassium chloride (KCl). This is achieved by replacing part of the phosphate buffer by a measured amount of 1.0 M potassium chloride (KCl) solution; the resulting $pK_{a(\text{app})}$ values are determined by both potentiometric and TBO metachromasy titration. Polyphosphate and κ -carrageenan are not investigated as the $pK_{a(\text{app})}$ values of these compounds are not assessable by titration. The results of the experiments are summarized in Table 4.

The results presented in Table 4 show that potentiometric titration generates slightly higher $pK_{a(\text{app})}$ values, but the observed trends at increasing ionic strength are comparable. Overall, as the ionic strength of the solution is increased, the $pK_{a(\text{app})}$ values decrease. The data for alginate, gellan and pectin show only a minor

decrease in $pK_{a(\text{app})}$ with increasing ionic strength up to 10 mM KCl, whereas NaCMC remains virtually constant (within the experimental error). NaPAA shows the strongest decrease in $pK_{a(\text{app})}$ with increased ionic strength, which seems sensible as this polymer has the smallest inter-charge distance of those tested, hence screening of weakly acid polyelectrolytes groups provided by additional ions would have the biggest impact on its protonation state.

The ionic strength region investigated is limited and seems insufficient to reduce the observed $pK_{a(\text{app})}$ values to the monomeric pK_a values, hence higher ionic strengths are required (>10 mM). To test this, experiments are performed using the same method of KCl addition for the metachromatic dye titration only. However, experiments at further elevated ionic strength (>10 mM KCl added) do not seem possible for the metachromatic titration method as the initial pre-formed complexes no longer exhibit metachromasy. The onset of TBO displacement is already apparent in the reported experiments up to 10 mM KCl added, as shown in Fig. 4.

As shown in Fig. 4, increasing ionic strength suppresses the magnitude of the ratio A_m/A_M causing curves to flatten, making curve fitting more susceptible to inaccuracy at high ionic strength. The decrease in the ratio A_m/A_M is mainly caused by a decrease in the metachromatic peak height A_m , whereas the monomer peak height A_M shows a slight increase. The explanation for these observations is the following. When the ionic strength is raised by the addition of KCl, the supplemented potassium ions engage in direct competition with TBO, thus displacing part of the dye from its stacked state and lowering the metachromatic absorption peak height (Yamaoka & Takatsuki, 1981). From the data presented here, the highest ionic strength that still allows for sufficient data quality for trend analysis is ≈ 10 mM. So, it is still possible to use TBO metachromasy to detect the effective pK_a as a function of the ionic strength, but its accuracy drops with increasing salt concentration.

3.4. Influence of initial polyelectrolyte–TBO complex composition on the determination of $pK_{a(\text{app})}$

To shorten the time required determining $pK_{a(\text{app})}$ values of weak polyelectrolytes with unknown stoichiometry, it would be beneficial to know if it is possible to skip the determination of stoichiometry, in other words does the determined $pK_{a(\text{app})}$ value depend on initial complex composition? To answer this question, pre-formed complexes of different composition (P/D) were made and titrated following the so far used approach for alginate. The resulting titration curves are summarized in Fig. 5.

The data listed in the inset of Fig. 5 reveal that the determined $pK_{a(\text{app})}$ value of non-stoichiometric alginate–TBO complexes, significantly deviates from the value established for a stoichiometric complex (at P/D = 1). Next to the deviation in $pK_{a(\text{app})}$ value it appears that the curves take up a different shape at P/D > 1, deviating from the sigmoidal curve obtained at P/D ≤ 1. Hence, a stoichiometric alginate–TBO complex is required for $pK_{a(\text{app})}$

Table 4
Influence of ionic strength, by addition of KCl, on $pK_{a(\text{app})}$ of polyelectrolytes.

Polyelectrolyte	Added KCl [mM]	pH Titration direction	$pK_{a(\text{app})}$	
			TBO-method	Pot. Titration
Alginate	0	high-to-low	4.40 ± 0.01	4.57 ± 0.05
	0	low-to-high	4.52 ± 0.02	–
	1	high-to-low	–	4.54 ± 0.05
	3	high-to-low	4.24 ± 0.03	4.43 ± 0.05
	5	high-to-low	4.23 ± 0.02	–
	10	high-to-low	4.07 ± 0.03	4.35 ± 0.04
Gellan	0	high-to-low	–	4.70 ± 0.07
	3	high-to-low	–	4.50 ± 0.17
	10	high-to-low	–	4.44 ± 0.14
NaCMC	0	high-to-low	4.58 ± 0.03	4.76 ± 0.05
	3	high-to-low	4.53 ± 0.02	4.78 ± 0.04
	5	high-to-low	4.60 ± 0.03	–
	10	high-to-low	–	4.73 ± 0.02
Pectin	0	high-to-low	–	4.65 ± 0.07
	3	high-to-low	–	4.61 ± 0.09
	10	high-to-low	–	4.52 ± 0.19
NaPAA	0	high-to-low	5.75 ± 0.10	5.40 ± 0.02
	3	high-to-low	5.46 ± 0.05	–
	10	high-to-low	4.66 ± 0.05	–

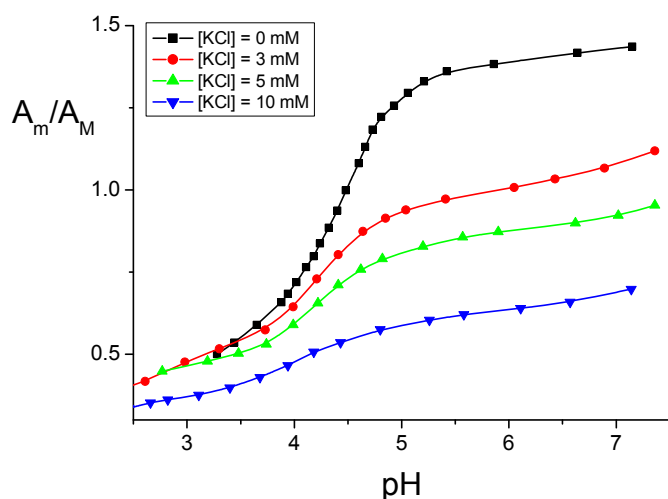


Fig. 4. TBO-method titration curves for alginate at different KCl concentrations added. The relevant absorption signal is the peak ratio A_m/A_M , versus pH. Overall medium conditions: 1 mM phosphate buffer at pH = 7.0.

determination.

To explain the deviations observed for non-stoichiometric complexes, two situations need to be examined: $P/D < 1$; with a relatively excess of TBO, and $P/D > 1$; with a relative excess of alginate binding sites. At $P/D < 1$, unbound TBO may compete with the added protons causing a higher concentration of protons required to displace bound TBO, i.e. a lower pH, which shifts the titration curve to lower pH values and hence a lower $pK_{a(\text{app})}$ is found. In case $P/D > 1$, the excess of binding sites allows TBO to bind to the most favourable sites. This also implies that not all binding sites on alginate are identical, as was already suggested by Draget, Moe, Skjåk-Bræk, and Smidsrød (2006, pp. 289–334). Fig. 5 shows that initial A_m/A_M values at neutral pH are higher at the same TBO concentration which corroborates the suggestion of diversity in binding sites. When protons are added they will first compete with binding sites that have no bound TBO but simple counter-ions such as sodium. This causes A_m/A_M to remain fairly constant at the highest pH values. Next, once these sites have bound a proton,

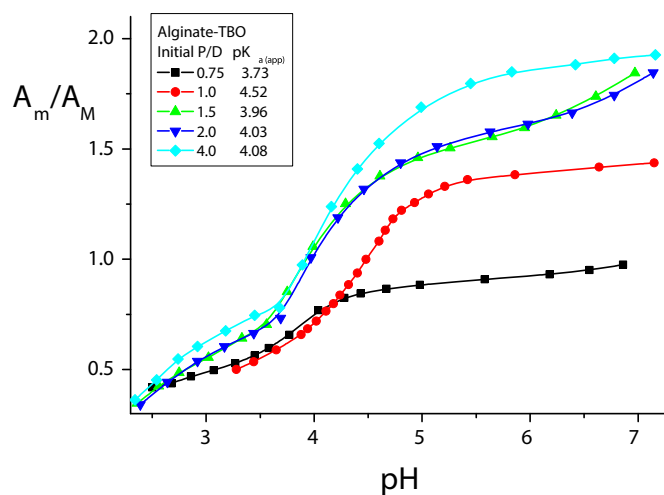


Fig. 5. TBO-method titration curves for alginate at different initial complex compositions. Peak ratio A_m/A_M , versus pH. (Solvent conditions phosphate buffer 1 mM, pH = 7.0).

displacement of TBO will start, following the same trend, as can be seen from the same slope in the curves, as at $P/D = 1$. This trend, however, continues towards a lower pH as explained by the competition with an excess of TBO, thus leading to a lower $pK_{a(\text{app})}$. Finally, all titration curves at complex stoichiometry $P/D > 1$ show an inflection point at lower pH. For this we presently have no explanation. More work, including other spectroscopic or scattering techniques, could clarify the type of organizations involved here.

4. Conclusions

Apparent pK_a ($pK_{a(\text{app})}$) values of food hydrocolloids are scarcely reported in spite of its importance in applications. As a result, researchers often have to resort to the use of the pK_a values of the monomer units for predicting specific, pH-related behaviour. Here it is shown that pH-titration of a metachromatic alginate-orthotoluidine blue complex yields a sigmoidal titration curve with its inflection point at the same pH value as the $pK_{a(\text{app})}$ value found by

potentiometric and surface tension titration. Methods to determine $pK_{a(\text{app})}$ values are abundant, however most methods are not applicable to food hydrocolloids at low concentration (<0.2 mM) and low solution ionic strength (≈ 1 mM). It is shown that micro-electrophoresis, a popular method reported in several publications, may give erroneous values if the neutralization of the food hydrocolloid is accompanied by aggregation or precipitation. The metachromatic dye titration method is evaluated for its use to determine $pK_{a(\text{app})}$ of five food hydrocolloids (alginate, pectin, sodium carboxymethylcellulose (NaCMC), κ -carrageenan and polyphosphate) and a common polyanion (sodium polyacrylate (NaPAA)) at low concentration (0.04–0.2 mM based on charged groups) and low ionic strength (1 mM phosphate buffer).

To apply the TBO metachromatic titration method successfully, TBO has to bind to the weak polyelectrolyte at such inter-charge distances that the dye ions can form stacks leading to metachromasy. For TBO, the inter-charge distance on the polyelectrolyte should not exceed 4.4 Å; for pectin and gellan this condition is not met. Alginate shows metachromasy even though the inter-charge distance based on molecular structure exceeds this value; it is therefore assumed that alginate is present in helical conformation. Next, the $pK_{a(\text{app})}$ values of all polymers are determined using metachromatic TBO, potentiometric and/or surface tension titration methods. In case multiple methods could be applied the data are in good agreement. The metachromatic TBO titration allows the determination of $pK_{a(\text{app})}$ values of sulphate (κ -carrageenan $pK_{a(\text{app})} = 1.2$), phosphate (poly(phosphate) $pK_{a(\text{app})} = 2.1$) and carboxylic acid groups (alginate $pK_{a(\text{app})} = 4.6$, NaCMC $pK_{a(\text{app})} = 4.6$ and NaPAA $pK_{a(\text{app})} = 5.8$). Experiments show that increasing the solution ionic strength by the addition of, up to 10 mM, potassium chloride (KCl) lowers the metachromasy intensity by displacement of TBO from its stacked binding. The $pK_{a(\text{app})}$ determined by metachromatic TBO and potentiometric titrations, up to 10 mM added KCl, show a slight decrease upon increasing the ionic strength. Data reported here suggest that ionic strength of approximately 10 mM KCl is the upper limit to obtain sufficient data quality to determine $pK_{a(\text{app})}$ by the presented method. Finally, for alginate it is shown that a stoichiometric alginate-TBO complex is required for $pK_{a(\text{app})}$ determination, in case of non-stoichiometric complexes lower values of $pK_{a(\text{app})}$ are obtained.

The presented metachromatic TBO titration method is a useful tool for the practical determination of $pK_{a(\text{app})}$ values of food hydrocolloids and other polymers in aqueous solutions, especially at low polyelectrolyte concentrations and in ionic strength up to 10 mM. It is realized that food hydrocolloids interact with other species in complex food matrices. Such other compounds may hamper the establishment of the metachromatic complex. In some cases some clean up may be required to properly analyse the apparent pK_a .

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Appendix A. Supplementary data

Supplementary data related to this article can be found at

<https://doi.org/10.1016/j.foodhyd.2018.02.049>.

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