Removal of anionic dyes in aqueous solution by flocculation with cellulose ampholytes

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ABSTRACT

Cellulose ampholytes (CAms) prepared from biocompatible and biodegradable carboxymethyl cellulose (CMC) could represent an environmentally friendly alternative for the efficient removal of anionic dyes from water effluents. In order to evaluate their potential for this application, a series of CAms with a degree of cationic substitution (DS_C) between 0.26 and 1.08 were prepared from sodium CMC, by cationization with 2,3-epoxypropyltrimethylammonium chloride. The flocculation ability of the CAms against the anionic dye Acid Red 13 (AR13) was found to strongly depend on the DS_C as well as on the pH of the solution. The highest flocculation ability was observed at lower pH and higher DS_C: a color removal as high as 99% was achieved at pH 3 using a CAm with the highest DS_C. The kinetic analysis revealed that the flocculation of AR13 can be accurately fitted by a pseudo-second order kinetic model, which thus allows to predict the flocculation behavior of CAms under these conditions. Besides AR13, the ampholytes also showed high flocculation ability against Acid Red 9 and Acid Blue 13. The flocculation behavior of these anionic dyes followed the Langmuir adsorption isotherm model, which revealed the maximum flocculation capacity of a CAm for these dyes. Moreover, the analysis of the flocculation isotherm data and the SEM observation of the formed flocs allowed to determine the flocculation mechanism.

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Nomenclature

CMC	Carboxymethyl cellulose
EPTMAC	Epoxypropyltrimethylammonium chloride
CAm	Cellulose ampholyte
DSS	4,4-Dimethyl-4-silapentane-1-sulfonic acid

1. Introduction

More than 7×10^5 ton of dyes are used annually in the chemical industry worldwide, about 10–15% of which are discharged into water as effluents, seriously polluting the environment and affecting human as well as aquatic organisms [1,2]. There are more than ten thousand types of commercially available dyes, most of which are considered toxic [1–3]. In addition, most dyes have complex and very stable aromatic structures, and thus tend to accumulate in nature [4–6]. Industrial treatments to remove dyes can involve oxidation [7,8], coagulation [8,9], flocculation [10,11], ion-exchange [12], photochemical destruction [13], and other methods. In particular, adsorption has been widely used for the removal of dyes from effluents, and activated carbon is conventionally used for adsorbing dyes from wastewaters. However, the use of activated carbon is limited by its relatively high cost and difficult regeneration [14].

Coagulation of dye-containing wastewater has been used for many years either as a pre-treatment or as the main method to treat wastewater due to its low capital cost [15]. Recently, much attention has been devoted to a coagulation-flocculation process for dye removal from water, which employs natural polysaccharides such as sodium alginate [16], carrageenan [17], pectin [18], carboxymethyl cellulose [19], chitosan [20], and carboxymethyl chitin [20]. These compounds are particularly attractive due to their biocompatibility, biodegradability, and anionic properties. However, even though they exhibited promising results, the use of these polysaccharides as anionic flocculation agents is still infrequent. The practical use of anionic polysaccharides as flocculation reagents is limited by their poor solubility in acidic solutions [21,22]. Chitosan, a cationic polysaccharide abundant in nature, exhibits excellent flocculation properties towards anionic dyes and suspended particles with negatively charged surface. The amino groups of chitosan are protonated and thus charged positively in acidic solutions, resulting in facile dissolution and excellent flocculation performance in acidic solutions [22]. On the other hand, the

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poor solubility in neutral and alkaline solutions prevent the use of chitosan as flocculant in these conditions [22].

Among the naturally occurring polysaccharides, cellulose – which is the most abundant natural polymer on earth – represents one of the most environmentally friendly non-food sources for the production of a wide range of eco-friendly products. The high degree of polymerization and number of potential chemical modifications of cellulose also make it an attractive candidate for flocculation purposes [23,24]. For example, sodium carboxymethyl cellulose (CMC), a well known water-soluble cellulose derivative with properties characteristic of an anionic polymer, has shown promising results as an anionic flocculation agent, despite the poor solubility in acidic solutions that limits its practical application as flocculation reagent in these conditions [19]. In addition, cationized cellulose, produced by homogeneous cationization with 2,3-epoxypropyltrimethylammonium chloride (EPTMAC), was reported to exhibit good cationic flocculation ability toward kaolin suspensions [25]. However, as cellulose is highly crystallized by a strong network of inter- and intra-molecular hydrogen bonds, its dissolution requires a time-consuming processing with unpopular solvents such as aqueous sodium hydroxide/urea solution, lithium chloride/dimethyl sulfoxide, tetra-n-butylammonium fluoride/dimethyl sulfoxide, and specific ionic liquids, and/or preswelling by sequential solvent change and heating [26]. In our previous work, cellulose ampholytes (CAms) containing both cationic quaternary ammonium sodium salt and anionic carboxymethyl groups were prepared from homogeneous etherification of CMC dissolved in aqueous solution in the presence of EPTMAC as cationic reagent. The starting CMC material is relatively cheap and easily dissolved in NaOH solution, and its unsubstituted hydroxyls can easily react with EPTMAC to form cationic groups. Thanks to these cationic groups, the obtained CAms could easily dissolve in solutions within a wide pH range and exhibited excellent flocculation performance toward suspended kaolin particles [27]. Similarly, it was reported that several polysaccharide-based amphoteric flocculants, including chitosan [28], amylopectin [29], and starch [30], showed enhanced solubility and good flocculation performance.

In this study, a series of CAms having a different degree of cationic substitution (DS$_C$) was prepared from CMC with a degree of anionic substitution (DS$_A$) of 0.60 (Fig. 1(A)), and the effects of several factors on the flocculation ability of the prepared CAms against the Acid Red 13 (AR13) anionic dye in aqueous solution were investigated in detail. In addition, in order to determine the mechanism of interaction between CAm and the anionic dyes during flocculation, we investigated the flocculation behavior of CAm towards Acid Red 9 (AR9), AR13, and Acid Blue 92 (AB92) anionic dyes, which contain 1–3 anionic sulfone groups per molecule, respectively (Fig. 1(B)).

2. Materials and methods

2.1. Materials

CMC (DS$_A$ = 0.60, molecular weight (Mw) = 2.6×10^5 g mol^{-1}) was kindly supplied from Dai-ichi Kogyo Seiyaku Co. (Japan). EPTMAC was purchased from Sigma–Aldrich (USA). AR9, AR13, and AB92 were all purchased from Tokyo Chemical Industry Co. Ltd. (Japan). The molecular weights of AR9, AR13, and AB92 were 400.4, 502.4, and 695.6 g mol^{-1}, respectively. Other chemicals were chemically pure grade and all solutions were prepared with pure water.

2.2. Synthesis of CAms

A series of four CAms (labeled **1**–**4**) were prepared from CMC with EPTMAC according to the method previously reported [27]. Briefly, CMC (1.0 g) was dissolved in 1.5 mmol L^{-1} NaOH solution at 277 K, and then EPTMAC (1.4 g) was added dropwise over a period of 20 min. After precipitation with acetone, the obtained precipitates were washed with a 1:1 mixture of acetone/distilled water to reach neutral pH, dried under vacuum, and then milled and filtered through a 40-mesh sieve to obtain CAm **1**. A similar method was used to prepare CAms **2**–**4**, by adapting the feed amount of EPTMAC, which was set to 3.5 g, 4.9 g, and 7.0 g for CAms **2**–**4**, respectively. All CAms were stored in a desiccator under vacuum until ready for use.

2.3. ^{13}C NMR spectroscopy

NMR spectra of the CAms were measured at 353 K in deuterium oxide containing 0.1% 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as an internal standard using an AV III 500 MHz spectrometer equipped with a 5 mm BBO probe (Bruker BIoSpin GmbH, Germany). The quantitative ^{13}C NMR spectra were obtained by the inverse-gated ^{1}H decoupling method [31] with a flip angle for ^{13}C excitation pulse of 30° and a pulse repetition time of 45 s [32]. The ^{13}C chemical shifts were calibrated by setting the methyl peak of DSS to 0 ppm.

2.4. Flocculation of anionic dyes

Three kinds of anionic dyes, whose structures are shown in Fig. 1(B), were used for the flocculation studies. A buffer solution (10 mL) containing each dye in a concentration range of 50–1600 mg L^{-1} was poured in a glass vial, and the CAm was added to the dye solution. The mixture was immediately placed in a shaker at 120 rpm and 298 K for 1 h, after which the suspension was left to settle for 5 min. The supernatant of the suspension was filtered by using a nylon membrane filter with a pore size of 0.45 μm (Rephile Bioscience, Ltd., USA). The dye concentration of the filtrate was determined by an Epoch 96 well micro-volume spectrophotometer (BioTek Instruments, Inc., USA) with a 96-wells microplate, using an earlier prepared calibration curve at 492 nm for AR9, 504 nm for AR13, and 572 nm for AB92. The percent removal of each dye and the flocculation ability (q_t) of each CAm at contact time t were calculated using Eqs. (1) and (2):

$$(\text{Dye removal})\% = \frac{C_0 - C}{C_0} \times 100 \tag{1}$$

$$q_t = (C_0 - C) \times \frac{V}{m} \tag{2}$$

where C_0 (mg L^{-1}) is the initial concentration of dye solution, C (mg L^{-1}) is the concentration of dye solution at contact time t, V (L) is the volume of dye solution, and m (g) is the mass of CAm. 20 mM citric acid-sodium citrate (pH 3–6), 20 mM NaH$_2$PO$_4$–Na$_2$HPO$_4$ (pH 7–8), 20 mM Tris-HCl (pH 9.0), and 20 mM NaHCO$_3$–NaOH (pH 10) were used as buffer solutions for the flocculation media.

2.5. SEM measurements

Scanning electron microscopy (SEM) images of the samples were obtained using a JSM 6330F scanning electron microscope (JEOL Ltd., Japan), using a potential of 1 kV and Au vapor deposition to prepare the sample surface.
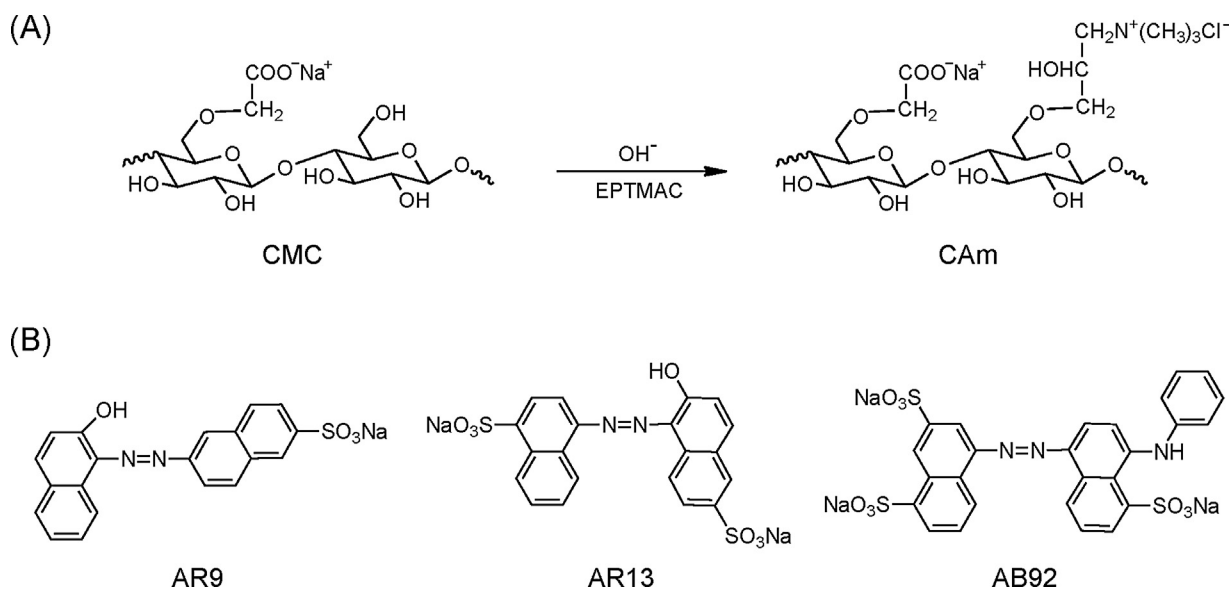
Fig. 1. (A) Scheme illustrating the preparation of CAm from CMC;(B) structures of the anionic dyes used in this study.The anionic carboxymethyl and the cationic quaternary ammonium chloride groups of the CAm scan be located at any hydroxyl group of the anhydro glucose unit of cellulose, but they are not shown in the figure.

Table 1
DS_C, DS_A, and yields of CAms **1–4**.

CAm	DS_C	DS_A	Yield (%)[a]
1	0.26	0.60	1.02 (89)
2	0.66	0.60	1.28 (90)
3	0.83	0.60	1.36 (88)
4	1.08	0.60	1.58 (92)

[a] Yield (%) was determined by Eq. (5).

3. Results and discussion

3.1. Structural characterization of CAms

The quantitative ^{13}C NMR spectra of CAms **1–4** in D_2O are shown in Fig. 2, which also highlights previous assignments of ^{13}C resonances [27]. As the EPTMAC feed amount used in the preparation of the CAms was increased, the intensity of the 57 ppm peak corresponding to the methyl carbon in the cationic groups was enhanced, while the signal of the carbonyl carbon in the anionic $-COO^-Na^+$ groups at 180 ppm hardly changed. The integrals of the ^{13}C signals for the methyl and carbonyl carbons ($I_{C=O}$ and I_{CH3}, respectively) and for the anomeric carbon at 105 ppm (I_{C1}) in each spectrum can be used to determine the DS_C and DS_A according to Eqs. (3) and (4):

$$DS_A = \frac{I_{C=O}}{I_{C1}} \tag{3}$$

$$DS_C = \frac{I_{CH3}}{(3 \times I_{C1})} \tag{4}$$

The DS_C and DS_A values can then be used to calculate the yield obtained for each CAm through Eqs. (5) and (6):

$$\text{Yield}(\%) = \frac{\text{yield(g)}}{Mw_{AGU}(gmol)} \times \frac{100}{4.6 \times 13^{-3}(gmol)} \tag{5}$$

where Mw_{AGU} is the average molecular weight of the anhydroglucose unit for each CAm, which can be calculated through Eq. (6):

$$MW_{AGU}(gmol^{-1}) = 162 + 151.5 \times DS_C + 80 \times DS_A$$

Table 1 summarizes the DS_C, DS_A, and the yield of each CAm. The DS_C values for CAms **1–4** were 0.26, 0.66, 0.83, and 1.08, respectively, while the DS_A of all CAms was 0.60, indicating that no

dissociation of anionic groups or ether exchange between anionic and cationic groups had occurred during the preparation of the CAms. The yields were in range of 88–92%. These data are in good agreement with values previously reported [27].

3.2. Effects of external parameters on AR13 flocculation

3.2.1. Effect of solution pH

Among the external parameters which can affect the flocculation efficiency, the pH of the flocculation system is the most important one [27–30]. In addition, the charge balance between cationic and anionic groups of the amphoteric flocculants may also have great influence on the flocculation efficiency [27–30]. Therefore, we assessed the effects of these parameters on the flocculation ability of CAms toward AR13.

Fig. 3(A) shows the flocculation ability (q) for each CAm, defined as the amount of flocs per gram of CAm, at various pH ranging between 3 and 10. All CAms showed a maximum flocculation ability at pH 3. The flocculation ability was sharply reduced when the pH was increased from 4 to 5, and then slowly decreased with further pH increases from 5 to 10. In addition, the flocculation ability in all pH solutions follows the order CAm **1** < CAm **2** < CAm **3** < CAm **4**. Taking into account that AR13 is present in anionic form in the pH range investigated, the attraction between each CAm and AR13 is the dominant force that generates the dye flocculation, and the above result can be attributed to the different structure of the CAms studied here, which in turn depends on the solution pH.

The pKa of the carboxymethyl groups of CMC was reported to be around 4.3 [33], and the same value is also attributed to the pKa of the anionic groups within the CAm chains. When the pH is set to lower than 4, the negative charges on the surface of the CAms vanish due to the protonation of their carboxylate groups, and thus each CAm acts as a cationic polymer with a specific DS_C value. The anionic dyes can then diffuse to the positively charged surface of the CAm chains by electrostatic attraction forces, so that their flocculation occurs via a charge neutralization mechanism. Therefore, the maximum flocculation ability of all CAms could be achieved at pH 3. On the other hand, when the pH of the solution is higher than 5, the surface of CAms bears both positive and negative charges, according to the DS_C and DS_A values of the polymer. In these conditions, intermolecular and intramolecular charge neutralization is consid-
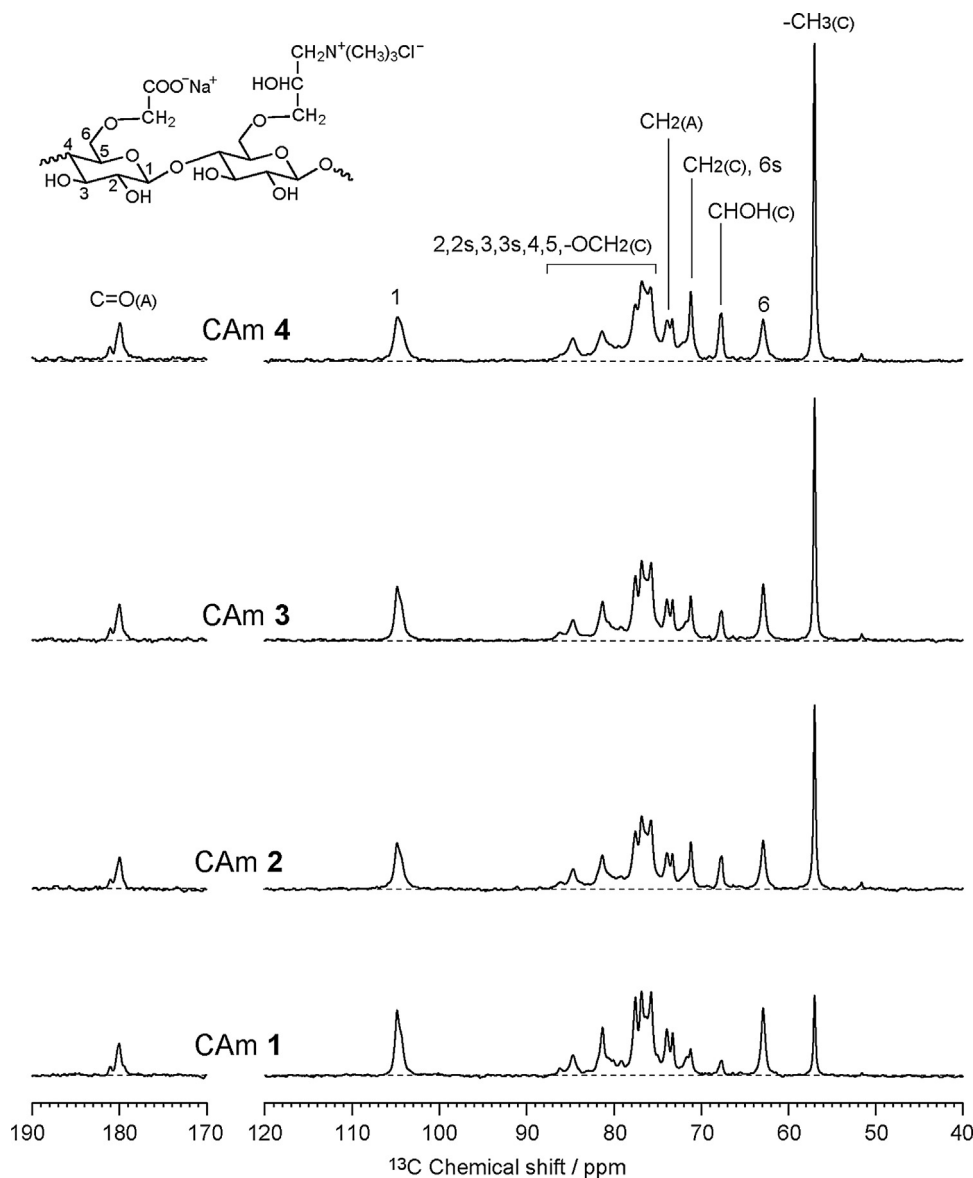
Fig. 2. ^{13}C NMR spectra of CAms in D2O. The resonance assignments are also shown in the figure. The C2s, C3s, C6s labels indicate C2, C3, and C6 centers in which hydroxyl groups are substituted by anionic and/or cationic groups, respectively. The subscripts (A) and (C) in the signal assignments denote anionic and cationic groups, respectively. The baseline for the spectra is shown as dotted lines.

ered to occur between the cationic groups and anionic groups of the CAm chains, suggesting that above pH 5, the effective cationic charge of each CAm should be considerably lower than the DSc of the polymer. Therefore, the drastic decrease in flocculation ability of the CAms with increasing solution pH is due to the lower cationic charge of the polymer surface, as well as to the electrostatic repulsion between the anionic charge of the polymer surface and the AR13 molecules. These results thus denote that, in order to flocculate the anionic dye, a combination of low pH of the flocculation medium and a high DSc of the CAm sample is preferable.

The effect of aggregation is considered in the floc formation from AR13 with CAms. The cationic groups of CAms bind to the anionic sites in AR13. Charge neutralization between an AR13 molecule and CAm chains enhanced the hydrophobicity of the formed polymer–dye complexes. As a result, hydrophobic interactions occur between the polymer–dye complex, which results in the aggregation of the complex and the formation of macro floc [34]. In addition, electrostatic interactions between the unneutralized cationic group of the polymer–dye complex and unneutralized

anionic group of the other complex is considered to prompt the aggregation.

3.2.2. Effect of temperature

The flocculation of AR13 by CAms **1**–**4** was investigated in the temperature range of 298–333 K, and the results are shown in Fig. 3(B). The flocculation ability of all CAms stays almost constant in the temperature range investigated, indicating that the temperature of the dye solution has no influence on the floc formation of AR13 with CAms. If the flocculation was affected by the temperature, then its mechanism would also involve physical adsorption, in addition to the electrostatic adsorption between the dye and CAm chains, because physical adsorption processes controlled by interactions such as Van der Waals forces generally exhibit a strong temperature dependence [35]. Therefore, this finding indicates that the floc formation can be described as a homogeneous adsorption process induced by the electrostatic interaction between the anionic dye and the cationic groups of the CAm chains.
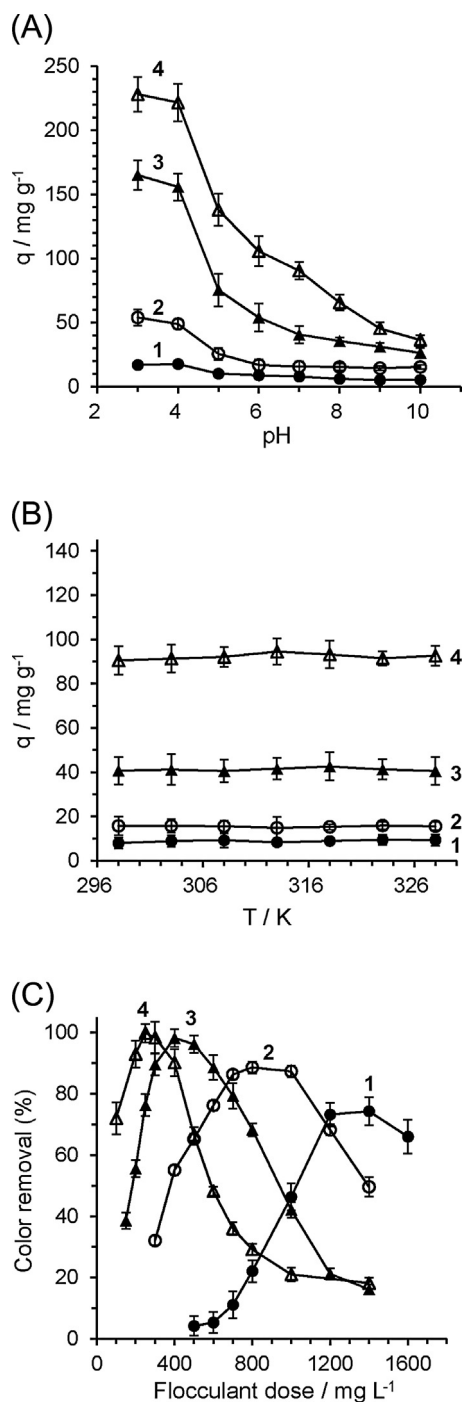
Fig. 3. (A) Effect of pH on the flocculation ability of AR13 by CAms.The initial concentration of the AR13 solution was 50 mg L^{-1}, and the amount of CAm added to the solution was 2 mg. (B) Effect of temperature on the flocculation of AR13 by CAms.The initial concentration of AR13 solution was 50 mg L^{-1},the amount of CAm added to the solution was 2 mg, and the pH of the buffer medium was 7. (C) Effect of the CAm **4** dosage on the AR13 flocculation at pH 3.The initial AR13 concentration was 50 mg L^{-1}.All experiments were performed with 10 mL of dye solution in a 22 mL glass vial.

3.2.3. Effect of the flocculant dose

To evaluate the effect of the CAm dose on the flocculation of AR13, CAms **1**–**4** with a variable flocculant dose (50–1600 mg L^{-1}) were exposed to a fixed initial dye concentration of AR13. The results in Fig. 3(C) show that the extent of color removal increased with increasing flocculant dose up to an optimal value, followed by a decreasing trend with further increases in dose. These curves

are typical of flocculant systems controlled by a charge neutralization mechanism [28–30,35]. In particular, when the amount of cationic charge was sufficient for the complete neutralization of the anionic charge, the color removal reached the maximum efficiency. Excess of cationic charge following overdosing of the flocculant induced a dispersion re-stabilization phenomenon and a subsequent decrease in the efficiency of the flocculation process [35].

As shown in Fig. 3(C), the DS$_C$ had strong influence on the optimal dose of flocculant: for CAms with high DS$_C$ values, the optimal color removal could be reached at lower flocculant doses. The optimum doses of CAm **1**–**4** were 250, 400, 800, and 1400 mg L^{-1}, respectively. The decrease in the optimal flocculant dose is due to the increase in the number of available sorption sites in the CAm chains. In addition, the maximum color removal of AR13 at the optimal dose for each CAm decreased with decreasing DS$_C$ of the polymer employed: the maximum color removal efficiency was 99.3% for CAm **4**, 97.9% for CAm **3**, 90.6% for CAm **2**, and 78.2% for CAm **1** at the optimal flocculant dosages. This suggests that a higher DSc could help to enhance the hydrophilicity of CAm in water, leading to increased dye binding due to higher accessibility of cationic groups along CAm chains [35].

From these observations, the most effective flocculant was determined to be CAm **4**, and the optimal conditions for AR13 removal by CAm **4** were pH 3 and a flocculant dosage of 250 mg L^{-1}. Under these optimal conditions, the AR13 removal efficiency was 99.3%. This efficiency is equal to or greater than that achieved using other coagulant/flocculation techniques for AR dye removal. For example, polyammonium chloride (PAC), which is a well-known inorganic polymer flocculant, exhibits excellent performance in the removal of various dyestuffs, including AR119, and the optimal pH, coagulation dosage, and initial AR119 concentration were 3.8, 57 mg L^{-1}, and 140 mgL^{-1}, respectively. Under these optimal conditions, the dye removal efficiency is 95.25% [36]. Therefore, although the optimal conditions for CAm **4** differ from those for PAC, the CAms with high DS$_C$ values are effective flocculants for the removal of anionic dyes.

3.3. Kinetic analysis

The contact time between flocculants and dye molecules is another important parameter for the flocculation processes because it can provide information on the flocculation kinetics of the dyes for a given initial concentration of flocculant [28–30]. Therefore, the effect of contact time on the flocculation ability of CAms **2**–**4** toward AR13 was investigated at pH 7, and the data are shown in Fig. 4(A). The flocculation behavior of AR13 by each CAm can be divided into three regions: the flocculation ability rapidly increased in the first 10 min, after which it gradually increased until equilibrium was reached at a contact time of 25 min, and then remained constant. The behavior in the first region is due to the rapid diffusion of the anionic dye molecules to the cationic groups on the surface of CAm chains and the subsequent charge neutralization. The second region, with a slower increase in flocculation ability, is due to the decrease of the effective cationic charge on the CAm surface. After 25 min into the third region, there was almost no further increase in adsorption of AR13 onto the CAm, indicating that the equilibrium time for the floc formation is about 25 min under the flocculation conditions investigated here.

Several kinetic models have been previously used to describe the kinetics of dye flocculation by polysaccharide-based flocculants [37]. Among these models, a pseudo-first order [38] and a pseudo second order kinetic model [39] were used to fit the experimental data for the flocculation of AR13 by CAms. The pseudo-first

Table 2
Kinetic parameters (Eqs. (7) and (8)) for flocculation of AR13 by CAms **2–4** at pH 7 and 298 K.

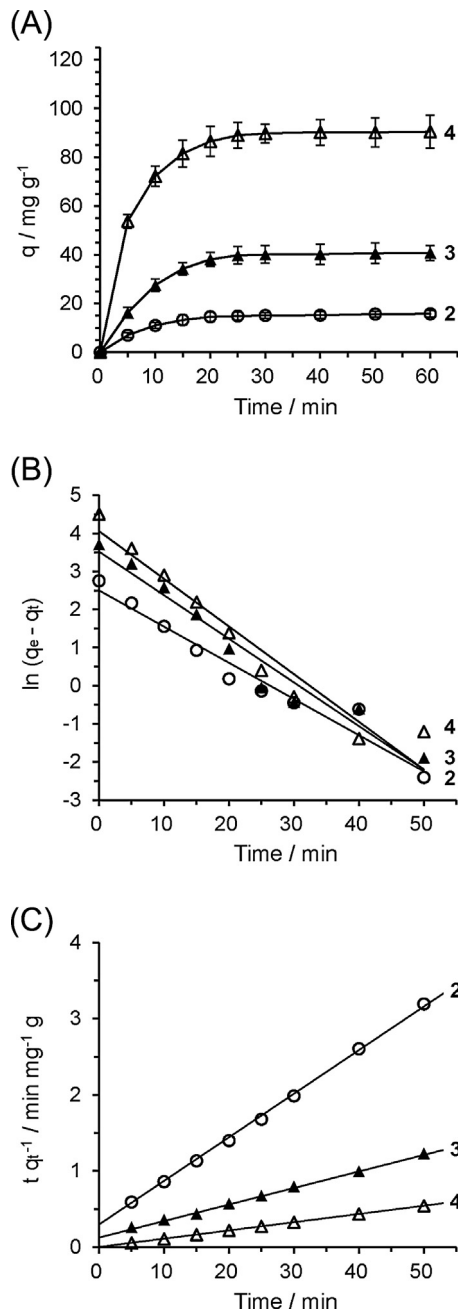| CAm	$q_{e,exp}$ /mg g^{-1}	Pseudo-first order model			Pseudo-second order model		
		k_1 / min^{-1}	$q_{e,cal}$ /mg g^{-1}	R^2	k_2 /g mg^{-1} min^{-1}	$q_{e,cal}$ /mg g^{-1}	R^2
2	16.0	0.069	8.42	0.928	0.059	16.7	0.999
3	41.2	0.056	18.0	0.803	0.023	43.7	0.998
4	92.0	0.066	35.2	0.811	0.010	95.8	0.999

Fig. 4. (A) Time dependence of flocculation of AR13 by CAms **2–4** at pH 7. The initial concentration of AR13 solution was 50 mg L^{-1}, and the volume of the dye solution was 10 mL. The amount of each CAm added to the solution was 2 mg. (B) Pseudo-first order kinetic plot and (C) pseudo-second order kinetic plot for the flocculation of AR13 by the CAms.

order and pseudo-second order kinetic models are expressed by the following formulas:

$$ln(q_e - q_t) = \ln(q_e) - k_1 t \tag{7}$$

$$\frac{t}{q_t} = k_2^{-1} q_e^{-1} + q_e^{-1} t \tag{8}$$

respectively, where k_1 and k_2 are the rate constants for the pseudo-first order and pseudo-second order kinetic models, while q_e and q_t are the flocculation abilities of each CAm at equilibrium and contact time t, respectively. The slopes and intercepts of $\ln(q_e - q_t)$ versus t plots were used to determine the pseudo-first order rate constant k_1 and the theoretical flocculation ability at equilibrium, $q_{e,cal}$. If the pseudo-second order model was applicable, the slopes and intercepts of plots of t/q_t versus t were used to determine the pseudo-second order rate constant k_2 and the corresponding theoretical flocculation ability at equilibrium $q_{e,cal}$ (Fig. 3(C)). The equilibrium time in the figure was set to 60 min, and for each CAm q_e was set to the q_t value at t = 60 min. The kinetic parameters for the two kinetic models determined from the curve-fitting plots are presented in Table 2. The correlation coefficients (R^2) for the pseudo-second order kinetic model are in the range of 0.998–0.999, considerably higher than those of the pseudo-first order model (R^2 = 0.803–0.923). Moreover, it was found that the theoretical $q_{e,cal}$ values determined by the pseudo-second order model were more consistent with the experimentally determined flocculation ability at equilibrium $q_{e,exp}$, compared with the $q_{e,cal}$ determined by the pseudo-first order kinetic model. This suggests that the pseudo-second order adsorption mechanism was predominant for the adsorption of the AR13 onto the CAms. A similar kinetic behavior has been reported for the electrostatic adsorption of ionic dyes onto polysaccharide-based flocculants [29,30,35,37]. Therefore, it is possible to predict the flocculation behavior of AR13 in the presence of CAms over the entire range of contact time by using the rate constant k_2 determined for the pseudo-second order kinetic model.

3.4. Flocculation isotherms

Fig. 5 shows the effect of the initial concentration (C_0) of AR9, AR13, and AB92 dyes on the flocculation ability at equilibrium (q_e) of CAm **3** at pH 3 and 7. For all cationic dyes, high q_e values were observed with increasing C_0, and saturation levels were gradually achieved at C_0 = 300 mg L^{-1}. In addition, the saturated q_e value at pH 7 was considerably lower than that at pH 3, suggesting that all cationic dyes were flocculated with the CAms via the charge neutralization mechanism.

Adsorption phenomena are usually described through isotherms, i.e., plots of the amount of adsorbate on the adsorbent as a function of pressure (in the case of a gas) or concentration (in the case of a liquid at constant temperature). The adsorption isotherm is important for identifying the interaction of the adsorbates on the adsorbents and for optimizing the use of adsorbents. Among a number of different isotherms, the Langmuir and Freundlich models are the most popular ones to describe the adsorption of a dye onto a polymer surface [40,41], and were applied here to
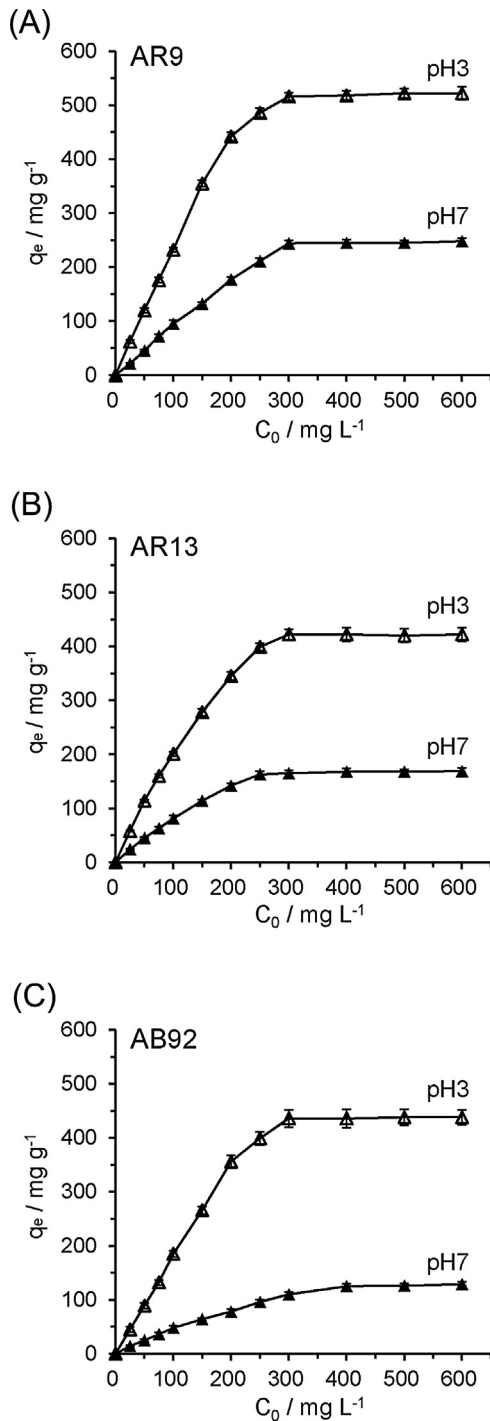
Fig. 5. (A) Effect of initial dye concentration (C_0) on the flocculation ability at equilibrium (q_e) of CAm **4** for (A) AR9, (B) AR13, and (C) AB92 at pH 3 and 7. All experiments were performed in 10 mL of each dye solution, and the amount of CAm added to the dye solution was 1 mg.

analyze the flocculation of cationic dyes AR9, AR13, and AB92 by the CAms.

The basic assumption of the Langmuir model is that adsorption occurs at specific homogeneous sites on the adsorbent. A further assumption is that once an adsorbate occupies a site, no further adsorption can take place at the same site [41]. The linear form of the Langmuir equation can be expressed by Eq. (9):

$$q_e^{-1} = Q_0^{-1} + (bQ_0C_e)^{-1} \qquad (9)$$

where q_e is the amount of adsorbate on the adsorbant at equilibrium (mg g^{-1}), Q_0 is the maximum monolayer coverage capacity (mg g^{-1}), b is the Langmuir isotherm constant (L mg^{-1}), and C_e is the equilibrium concentration of adsorbate (mg L^{-1}). Moreover, a dimensionless constant commonly known as separation factor (R_L), as defined by Weber and Chakravorti [42], can be defined as:

$$R_L = (1 + bC_0)^{-1} \qquad (10)$$

Based on the R_L values, the adsorption process can be categorized as unfavorable ($R_L > 1$), linear ($R_L = 1$), favorable ($0 < R_L < 1$), or irreversible ($R_L = 0$). On the other hand, the linearized form of the Freundlich isotherm equation is given by Eq. (11) [41]:

$$\ln q_e = \ln K_F + n^{-1}\ln C_e \qquad (11)$$

where K_F is the Freundlich isotherm constant, which is related to the adsorption capacity, and the dimensionless constant n^{-1} is an empirical parameter, which gives valuable information on the isotherm shape. Based on the n^{-1} values, the adsorption process may be classified as irreversible ($n^{-1} = 0$), favorable ($0 < n^{-1} < 1$), or unfavorable ($n^{-1} > 1$).

Based on the data in Fig. 5, linear plots corresponding to the Langmuir and Freundlich isotherm models are presented in Fig. 6, and the linearized isotherm coefficients of these models, as determined from the plots, are summarized in Table 3. As observed in Fig. 6 and Table 3, the dimensionless constants R_L obtained from the Langmuir model are in the 0.115–0.255 range, indicating favorable adsorption of all anionic dyes onto CAms. Additionally, in the case of the Freundlich model, the empirical constants n^{-1} lie in the range of 0.655–0.908, also indicating favorable adsorption of the dyes onto CAms. However, the correlation coefficients R^2 for the Langmuir model are in range of 0.996–0.999, while the Freundlich model gives lower values, between 0.942 and 0.991. Additionally, as shown in Table 3, the maximum monolayer coverage capacities (Q_0) of CAm **4** for AR9, AR13, and AB92 at pH 3 are 558 mg g^{-1}, 450 mg g^{-1}, and 457 mg g^{-1}, and those at pH 7 are 270 mg g^{-1}, 195 mg g^{-1}, and 140 mg g^{-1}, respectively. These data are in good agreement with the equilibrium flocculation ability of CAm **4** at the initial concentration of 600 mg L^{-1} shown in Fig. 5. They also imply that the adsorption of all anionic dyes onto CAm follows a Langmuir-type adsorption mechanism. The Langmuir constant b, which describes the desorption process of an anionic dye adsorbed on a CAm is of the order of 10^{-2}–10^{-3}, indicating that the flocs formed from anionic dyes by the CAm are very stable in solution.

The Langmuir adsorption isotherm model is based on the following assumptions, although some exceptions have been reported [43]:

- The adsorbent surface is uniform, i.e., all adsorption sites are equivalent.
- The adsorbed molecules do not interact with each other.
- All molecules are adsorbed by the same mechanism.
- At the maximum adsorption, only a monolayer is formed. In other words, the adsorbate molecules do not deposit on other molecules previously adsorbed on the adsorbent.

Therefore, based on the fact that our data fit well the Langmuir model, it can be concluded that the flocculation of dye molecules follows a homogeneous mechanism based on the charge neutralization between dye molecules and the cationic surface charge of CAm. This suggests that hydrophobic interactions between the dye molecules, for example π–π stacking interactions [44] between aromatic rings of cationic dyes, are small enough to be negligible, compared with the electrostatic interaction between the cationic dye and anionic CAm surface. If other interactions besides the electrostatic ones played a role in the flocculation of anionic dye, then
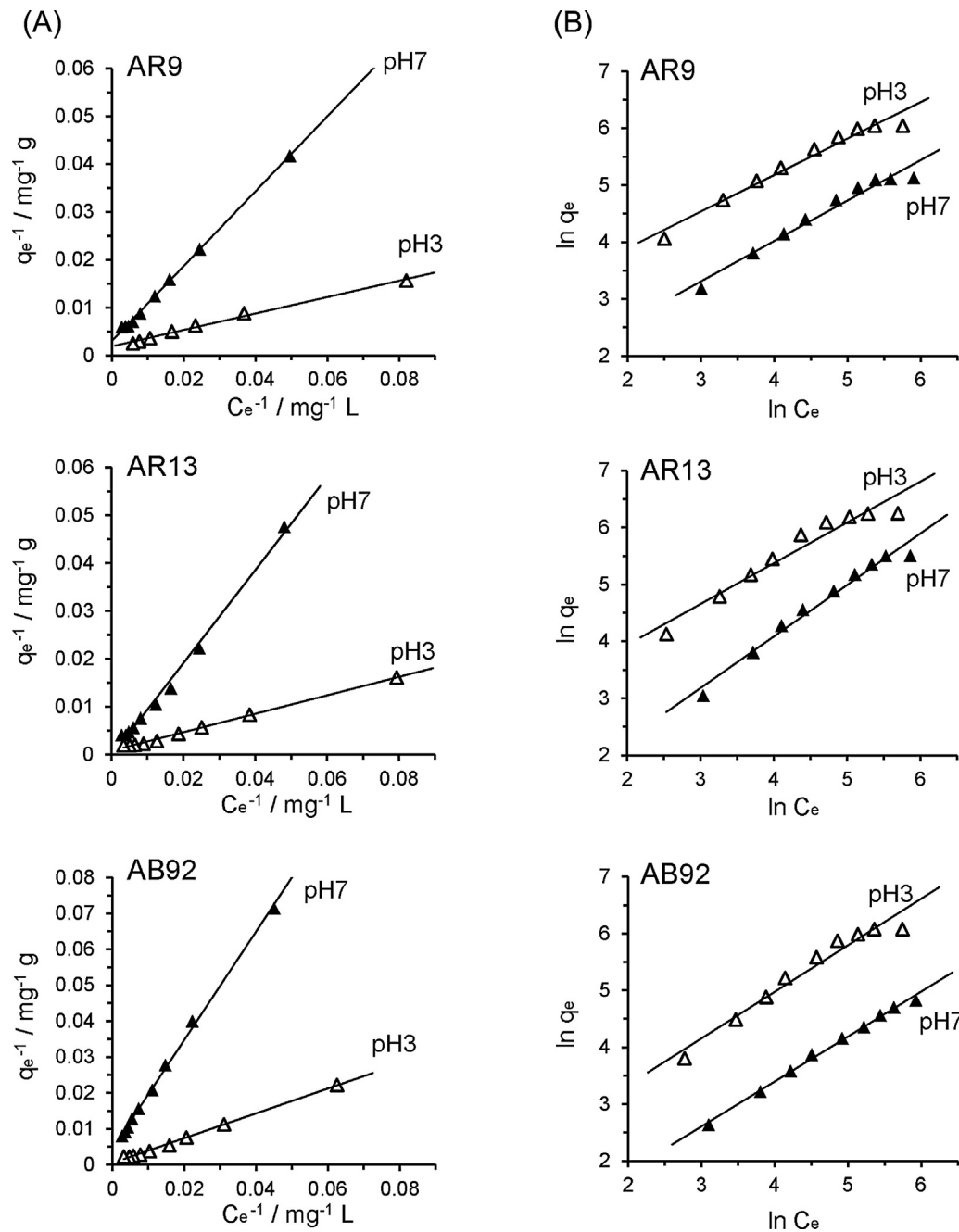
(A)

(B)

Fig.6. (A) Langmuir isotherm and (B) Freundlich isotherm for the flocculation of AR9, AR13, and AB92 at pH 3 and 7 using CAm **4** as a flocculant.

Table 3
Langmuir and Freundlich adsorption isotherm coefficients (Eqs. (9) and (11)) for the flocculation of cationic dyes by CAm **4** at pH 3 and 7.

Dye	pH	Langmuir adsorption isotherm				Fleundlich adsorption isotherm		
		C_0 / mg g^{-1}	b / mg^{-1} L	R_L	R^2	K_F / mg L^{-1}	n^{-1}	R^2
AR9	3	558	1.13×10^{-2}	0.128	0.996	12.2	0.719	0.963
	7	270	5.10×10^{-3}	0.246	0.998	1.57	0.908	0.966
AR13	3	450	1.29×10^{-2}	0.115	0.997	12.8	0.655	0.942
	7	195	6.64×10^{-3}	0.201	0.997	3.24	0.711	0.977
AB92	3	457	6.33×10^{-3}	0.208	0.998	5.12	0.820	0.957
	7	140	4.62×10^{-3}	0.255	0.998	1.26	0.791	0.991

the flocculation isotherm would be closer to the Freundlich adsorption isotherm model, which is generally employed to describe heterogeneous adsorption systems [40].

3.5. SEM analysis

Finally, the flocculation of AR9, AR13, and AB92 by CAm **4** was performed at pH 3 (Fig. 7A), and the formed floc was freeze-

(A)

AR9   AR13   AB92
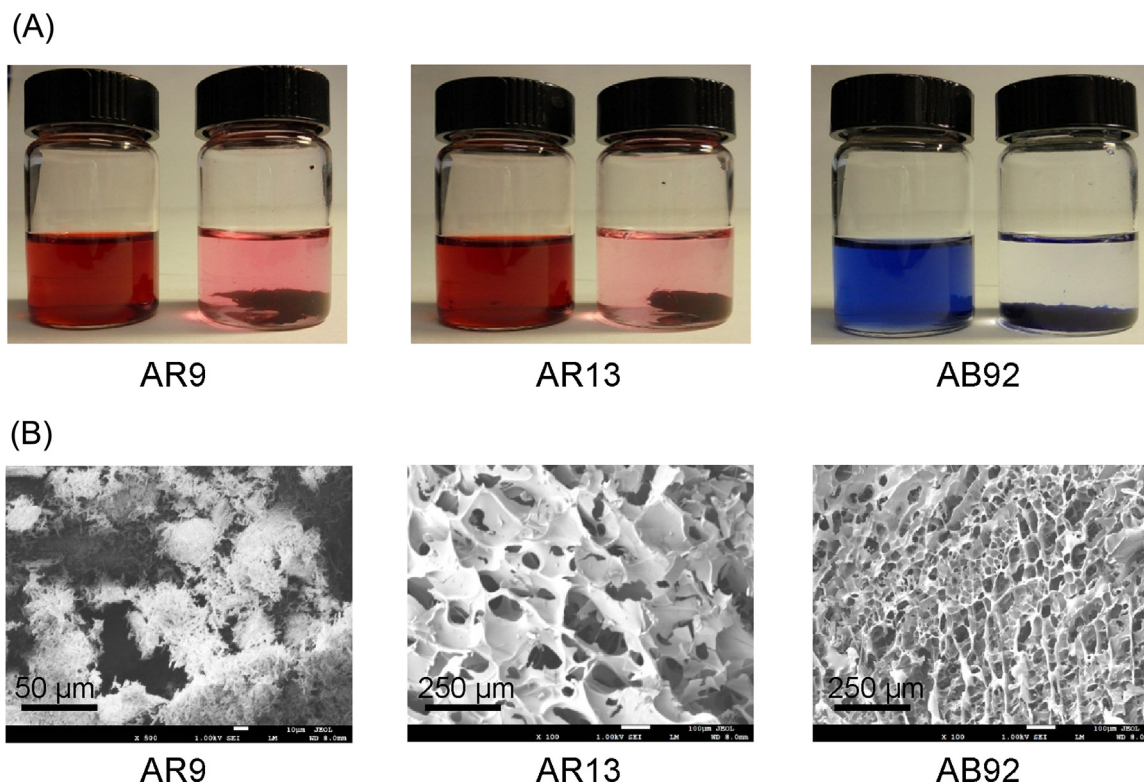
(B)

AR9   AR13   AB92

Fig. 7. (A) Photographs of AR9, AR13, and AB92 solutions before and after flocculation by CAm **4** at pH 3, and (B) SEM image of the flocs formed from these anionic dyes.

dried and then observed by SEM (Fig. 7B). The morphologies of the flocs formed from AR9 were completely different from those formed from AR13 and AB92. The flocs flocculated from AR9 contained small linear particles, which aggregated with each other to form the large flocs. On the other hand, flocs formed from AR13 and AB92 exhibited well-developed porous structures, with pore sizes of about 50–200 μm and 10–60 μm for AR13 and AB92, respectively. Such porous network structures have been frequently observed in the morphology of crosslinked polymers such as hydrogels: for example, hydrogels prepared from cellulose [45], carboxymethyl cellulose [33], chitosan [46], chitin [47], and guar gum [48], crosslinked by bifunctional crosslinking agents, showed similar porous morphologies. As described above, all polymer surfaces are positively charged at pH 3 due to the protonation of anionic carboxymethyl groups, and thus the floc formation takes place through the charge neutralization mechanism between the anionic dye and the positively charged polymer surface. Therefore, it can be suggested that the morphological differences between the flocs formed from AR9, AR13, and AB92 reflect the structural differences between AR9, AR13, and AB13 molecules, each containing 1–3 anionic sulfone groups, respectively.

In the case of flocculation of AR9 at pH 3, the AR9 molecule diffuses to neutralize the positively charged surface of a CAm chain. As the charge neutralization progresses, the polymer surface loses the cationic character, while its hydrophobicity sharply increases due to the surface being covered by AR9. Finally, as the polymer surfaces becomes neutral, polymer deposition occurs in the solution, and the hydrophobic interaction between the polymer chains leads the polymers to aggregate to each other to form the macroflocs, visible in the SEM photograph in Fig 7. Similarly to the floc formation from AR9, floc formation from AR13 and AB92 also involved the sulfone group of each dye molecule interacting with a cationic group on the CAm surface and neutralizing the corresponding charge. The remaining unreacted sulfone group(s) of the AR13 or AB92 molecules can also readily react with the other cationic groups on

the polymer surface. In particular, the sulfone group(s) of the dye attached on the CAm chain could interact with the same polymer chain, resulting in the formation of intramolecular crosslinks in the CAm mediated by the dye molecule. On the other hand, if the sulfone group(s) of the anionic dye interact with the other CAm chains, intermolecular crosslinking between CAm chains mediated by dye molecules can occur, resulting in a highly interconnected network structure. The morphology of flocs formed from AR13 and AB92 (Fig. 7) suggests that the porous structures were formed through the intra- and intermolecular crosslinking of CAm chains via the anionic dyes. In addition, it can be argued that the different pore sizes observed for the network structure of flocs formed from AR13 and from AB92 result from the different number of anionic sulfonic groups per molecule: AR13 has two sulfonic groups able to connect two chains of CAm via intermolecular crosslinking, while AB13 can crosslink up to three CAm chains through the three sulfonic groups in its structure. With respect to the proportion of intra- versus intermolecular crosslinking in the flocs formed from AR13 and AB92, the rather diluted initial concentration of CAm is considered to favor the formation of intramolecular rather than intermolecular crosslinking, although their relative exact proportions could not be determined only from the morphological observation of the flocs.

4. Conclusions

In order to assess the potential performance and suitability of cellulose ampholytes in treatments to remove dyes from water effluents, in this study we investigated the flocculation ability of various CAms obtained from CMC towards anionic dyes. The main findings were the following:

1. The flocculation ability strongly depends on the DS_C of the CAm used as a flocculant and on the pH of the flocculation medium (Fig. 3). In particular, a high flocculation efficiency can be achieved by flocculation at lower pH and using CAms having

high DS_C. The maximum color removal efficiency measured was 99.2% for AR13 at pH 3.

2. Flocculation of AR13 by CAms proceeds by a pseudo-second order kinetic mechanism (Fig. 4).
3. The flocculation of AR9, AR13, and AB13 followed the Langmuir, rather than Freundlich, adsorption isotherm model (Fig. 6), suggesting that the flocculation of these cationic dyes was predominantly controlled by a charge neutralization mechanism, and that physical (such as hydrophobic) interactions between the dye molecules were negligible.

Based on these findings, it can be concluded that the CAms obtained in this study had high flocculation ability towards anionic dies and may be suitable flocculants to readily remove anionic dies in solution. In addition, since the starting CMC material is nontoxic and biocompatibie [49], these CAms are also environmentally and biologically safe, thus they represent strong candidates as synthetic polymer flocculants for dye removal from effluents.

Acknowledgements

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