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Antimicrobial cationic dyes: part 2—thermal and hydrolytic stability

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Abstract

The thermal stability of antimicrobial cationic dyes was studied by differential scanning calorimetry (DSC) and thermogravimetric (TGA) analysis, and their hydrolytic stability was investigated by exposing the dyes in water under different pH conditions and analyzing hydrolyzed products by using electron absorption spectra and NMR analysis. These cationic antimicrobial dyes were stable below 190 °C, but higher temperature led to a two-step thermal decomposition. Both the mono-substituted and the bi-substituted dyes showed higher stability in acidic solutions than in basic conditions, and the highest stability was observed at pH 3. These unexpected results were believed to be related to the unique surfactant features and the positively charged hydrophilic components in the dyes. The structure effects on the stability of the dyes were discussed in detail.

Keywords: Thermal and hydrolytic stability; Antimicrobial dyes; Quaternary ammonium salts; Anthraquinones

1. Introduction

Antimicrobial cationic dyes were synthesized, and their antimicrobial functions and color effect in dye solutions were confirmed [1]. These dyes demonstrate both coloration and performance functions. To be suitable for application the dyes should withstand the conditions encountered in dyeing or wet treatment processes and in the subsequent use in different environment. Dyes can decompose thermally or hydrolytically and may break-down into colorless compounds or different colored compounds [2]. The two major dyeing procedures employed are exhaustion dyeing and pad-dry-cure. In both cases, inorganic salts, pH buffers and other auxiliaries are employed in dyeing baths, and higher temperatures and varied dyeing times are necessary. More specifically, the pH is a key factor in both methods, while a high temperature is also required in the pad-dry-cure process. Based on these considerations, dye stabilities under different pH values and temperature are of great interest in order to determine the optimum conditions for application of the dyes.

Cationic dyes are widely used in the dyeing of acrylic fibers and other cationic dyeable fibers. In exhaustion dyeing of acrylic materials, the normal

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pH range of dye bath is 3.5–6.0, and the temperature is usually 85–100 °C [3]. In the "pad-dry-cure" dyeing method, a temperature higher than 100 °C may be needed to enhance the interactions between dyes and fabrics. The exhaustion process is more popular. In both cases, cationic dyes are expected to withstand high temperatures as well as acidic pH conditions.

In our previous paper, we have reported the design, synthesis, and characterization of two series of antimicrobial cationic dyes [1]. This paper focuses on an investigation of thermal and hydrolytic stabilities of the dyes under different pH conditions and temperatures. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were employed to study the thermal stability, and electron absorption spectra were used to trace the hydrolytic stability of dyes.

2. Experimental

2.1. Thermal stability

Six antimicrobial cationic dyes were synthesized and purified by following the procedures described in our previous paper [1]. Three mono-substituted dyes are called m-4, m-8, and m-12, while bi-substituted ones are called bi-4, bi-8, and bi-12, based on the length of alkyl groups. The structures are shown in Fig. 1. Thermal analyses of the dyes were performed by using a differential scanning calorimeter (Shimadzu DSC-50) and a thermal gravimetric analyzer (Shimadzu TGA-50), respectively. The dye samples were heated at 10 °C/min and nitrogen gas was chosen as the atmosphere.

2.2. Hydrolysis analysis

The dyes were dissolved in aqueous solutions with a concentration of 0.4–1 mMol/L specified in each experiment, and the solutions were adjusted to various pH values by using sulfuric acid, acetic acid, sodium acetate, sodium bicarbonate or sodium carbonate, respectively. The solutions were heated to the required temperatures. At different time intervals, solution samples were taken and measured by a HITACHI U-2000 spectrophotometer to obtain the UV–vis adsorption spectra. The results were compared with the spectra of original dye solutions to determine the stability of dyes.

After hydrolysis, the precipitants formed in the dye solutions were filtered and thoroughly washed with deionized water. The resultant precipitants were further recrystallized by acetone for NMR analysis. NMR spectra were recorded on a Varian Inova 400 spectrometer. The residual filtrate was extracted by chloroform; the aqueous part was dried under vacuum at room temperature and also analyzed by NMR.

3. Results and discussion

3.1. Thermal stability

The cationic dyes contain anthraquinone and quaternary ammonium salt structures, and are thermally stable. In DSC spectra of mono-substituted dyes (Fig. 2), there were no obvious changes observed up to 190 °C, suggesting that the dyes are stable below this temperature. Above 190 °C, all dyes exhibited a sharp endothermic peak centered around 205–219 °C. Afterward, a wide exothermic peak in the range of 320–440 °C was detected. TGA analysis (Fig. 3) of the same samples indicated that these peaks corresponded to the decomposition of the dyes. This thermal decomposition involved two steps. The first step occurred in the temperature range of 200–240 °C, representing a weight loss of more than 25%. The second step ended at 400 °C, leading to carbonization of the products. Higher than 400 °C, no further weight loss could be detected. The total weight losses were more than 70%.

In general, anthraquinone structures are thermally stable [4]. Thus the decompositions of the dyes should be caused by their quaternary ammonium structures. The thermal stabilities of quaternary ammonium compounds (QAS) have been investigated elsewhere, and the first stage of the thermal decomposition of QAS is a dealkylation reaction, caused by a nucleophilic substitution reaction of the halide ion (X^-) at the α carbon center [5]. The whole process can be regarded as

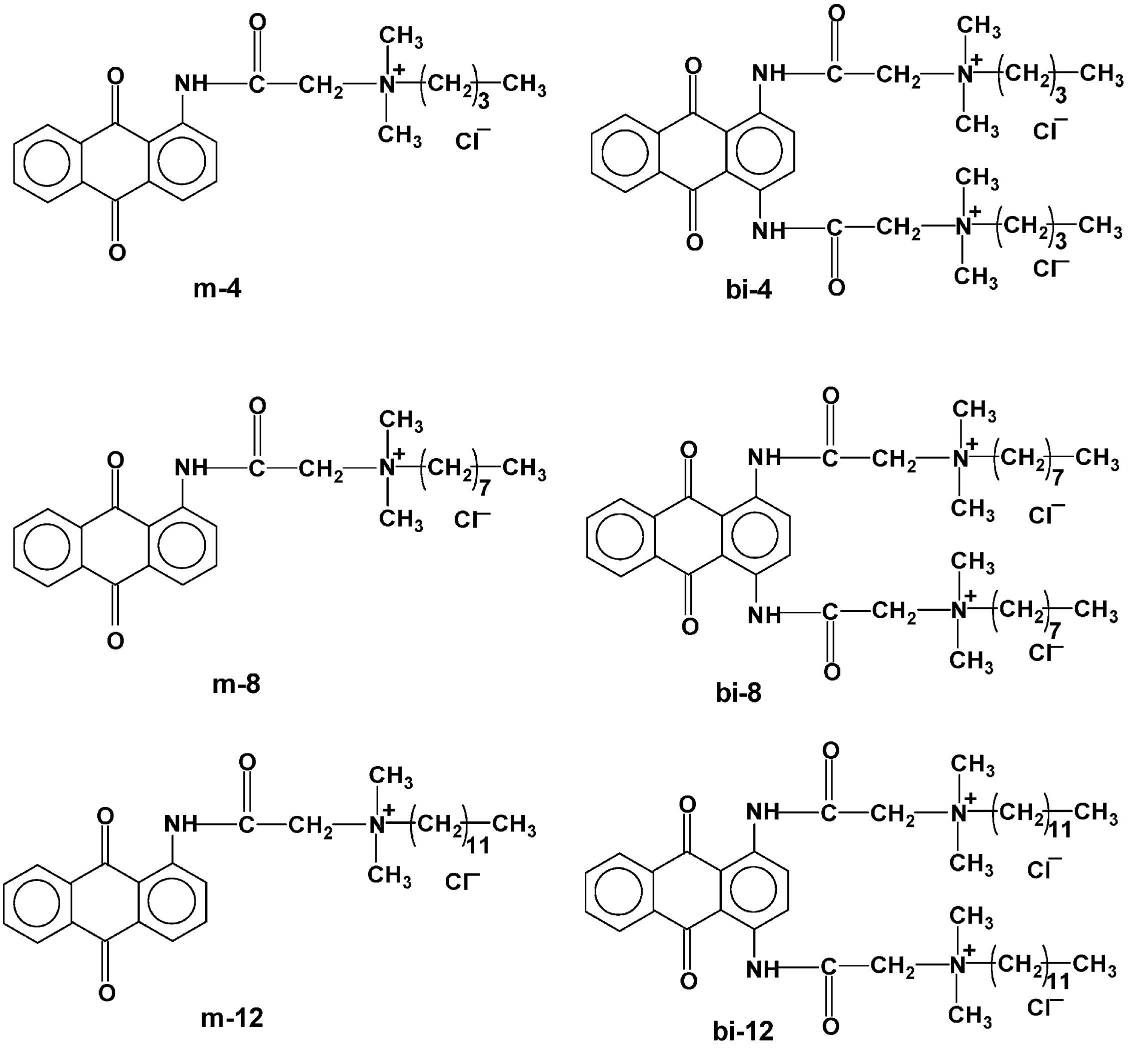


Fig. 1. Structures of the antimicrobial cationic dyes.

the reverse of quaternization of tertiary amines [6,7]. It should be mentioned that the attack of X^- to the most positive carbon atom is a thermodynamically favored reaction [6]. However, in thermal degradations, because of the high reaction temperatures, the selectivity of the substitution may be significantly reduced, and various substituted products can be obtained. The emission of these newly formed alkyl chloride or amine compounds accounts for the initial weight loss of the samples at 200 °C. The exothermic peak of the second step of the weight loss in the TGA curves is most likely attributed to the decomposition of the anthraquinone dye structures. Again, because of the same decomposition mechanisms, the DSC and TGA curves of the samples have very similar patterns in this range.

Similar phenomena can be observed in the thermal analysis of bi-substituted dyes. All the results indicate that the synthesized dyes are stable under 190 °C, which is sufficient for dyeing applications.

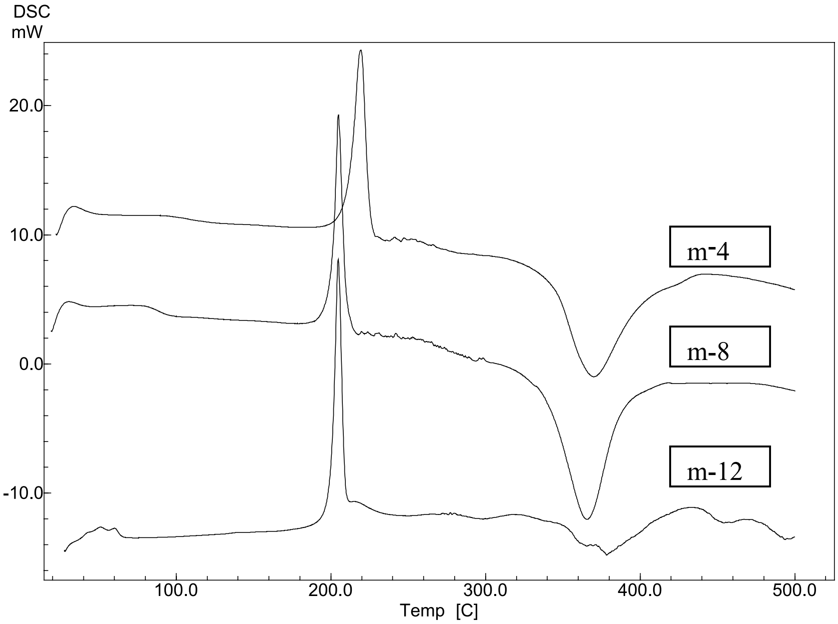


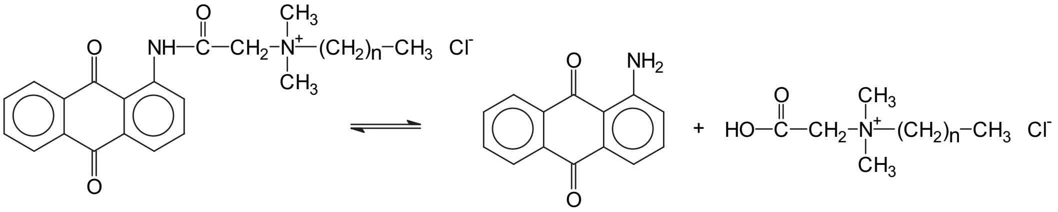
Fig. 2. DSC curves of the mono-substituted dyes.

3.2. Hydrolysis of dyes

The hydrolysis stability of the mono-substituted dyes was examined with a UV–visible spectrometer. As an example, adsorption spectra of m-4 dye solution at 100 °C and pH 5 for different times are shown in Fig. 4. The m-4 dye solution (0.4 mMol/L) was stable for about 6 h, evidenced by the almost unchanged visible spectra. After 8 h, however, the absorbance of the dye solution decreased dramatically, and this trend worsened after 10 h. Accompanying the decrease of absorbance, a red precipitant was also observed in the solution. ^{1}H NMR analysis of the precipitant suggests that 1-amino anthraquinone was produced as one of the hydrolysis products of m-4, as shown in Fig. 5. Theoretically, aromatic amine protons have chemical shifts around 3.0–5.0 ppm [8]: however, because hydrogen bonding may be formed between the amine and the trace water present in the sample, the peak of amine protons may be shifted and overlapped with other peaks. Therefore, in Fig. 5, proton H_h cannot be detected. In fact, in the ^{1}H NMR standard spectrum of 1-amino anthraquinone, the amine protons appear at 7.98 ppm, overlapping with protons H_e and H_f [9].

On the other hand, when the filtrate solution of the hydrolyzed m-4 dye was dried under vacuum, a solid compound was obtained. Both ^{1}H NMR and ^{13}C NMR spectra of the compound were measured (Figs. 6 and 7). The resultant is confirmed as carboxylmethyl-butyl-dimethyl ammonium chloride (CBDAC). The confirmation of both 1-amino-anthroquinon and CBDAC suggested that the hydrolysis of m-4 is caused by the cleavage of the amide linkage. In the hydrolysis study of m-8 and m-12, similar results were also obtained. Thus the hydrolysis reaction of the cationic dyes can be expressed in Eq. (1).

The hydrolytic stability of the bi-substituted dyes, bi-4, was conducted at pH 5 and 100 °C (0.5 mMol/L), and a purple precipitant was observed as one of the hydrolysis products. The NMR analysis proved that 1,4-diamino anthraquinone was the precipitant, and the spectrum is shown in Fig. 8. Similarly, CBDAC was also found as in the filtrate solution of the hydrolyzed bi-4. Both bi-8 and bi-12 also exhibited the formation of 1,4-diamino anthraquinone and the corresponding carboxylalkyl ammonium chlorides, indicating that bi-substituted dyes also undergo hydrolysis through the cleavage of amide linkages.



(1)

Mono-substituted dyes

n = 3:m-4
n = 7:m-8
n = 11:m-12

1-amino-anthraquinone

Alkylbetaine

n = 3: butylbetaine
n = 7: octylbetaine
n =11: dodecylbetaine

3.3. *Effect of pH*

The amide linkage between anthraquinone and quaternary ammonium salts is vulnerable to hydrolysis. Different pH conditions may affect the hydrolysis as well. Fig. 9 shows the period of stabilities of the mono-substituted dye solutions at different pH. Longer time indicates better stabilities of the dye under a specific pH condition. All dyes exhibited the highest stabilities at pH 3, a strong acidic condition. Amide hydrolysis was extensively studied by many researchers [10–16]. They found that amide linkages were susceptible to acid or base attacks, and the hydrolysis mechanisms were well established. Under acidic conditions, the hydrolysis of amides begins with the formation of *O*-protonated amides, followed by the break-down of the tetrahedral intermediate [15,16]. In basic solutions, however, the hydrolysis mechanism is nucleophilic substitution [10,11]. On the other hand, amides are regarded stable under neutral conditions.

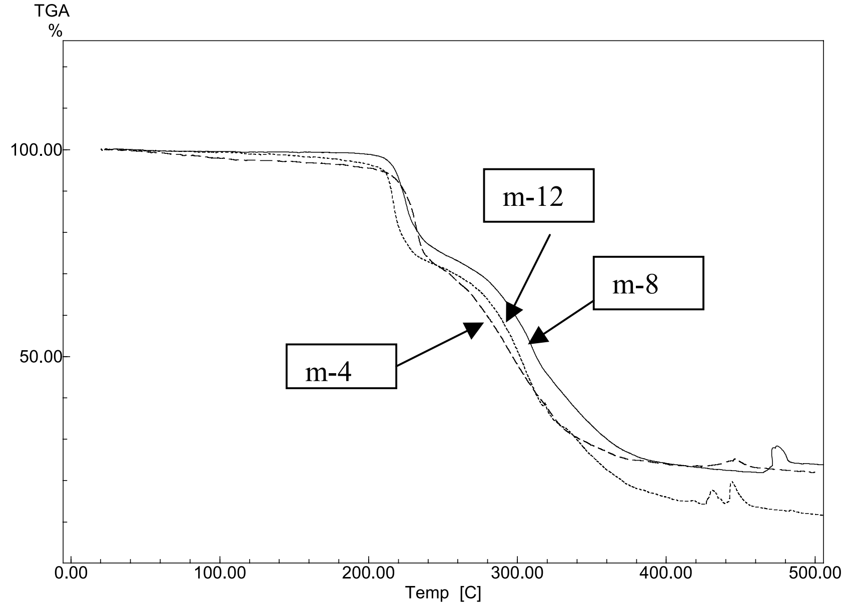


Fig. 3. TGA curves of the mono-substituted dyes.

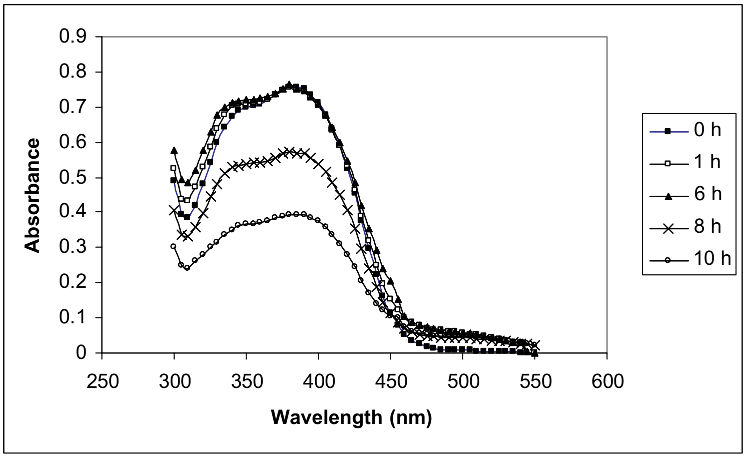


Fig. 4. UV–vis absorption spectra of m-4 at pH 5 and 100 °C (original dye concentration: 0.4 mMol/L).

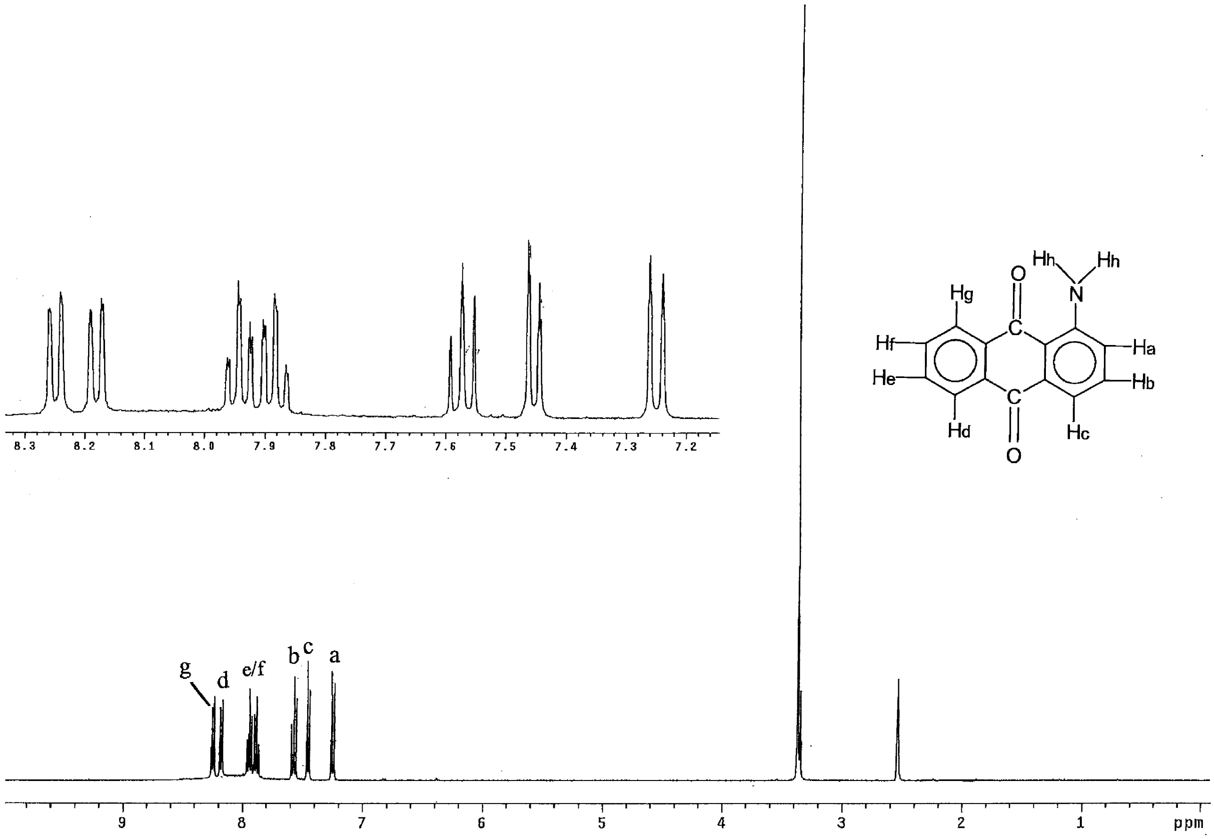


Fig. 5. ^{1}H NMR spectrum of 1-amino anthraquinone (solvent: DMSO-d_6).

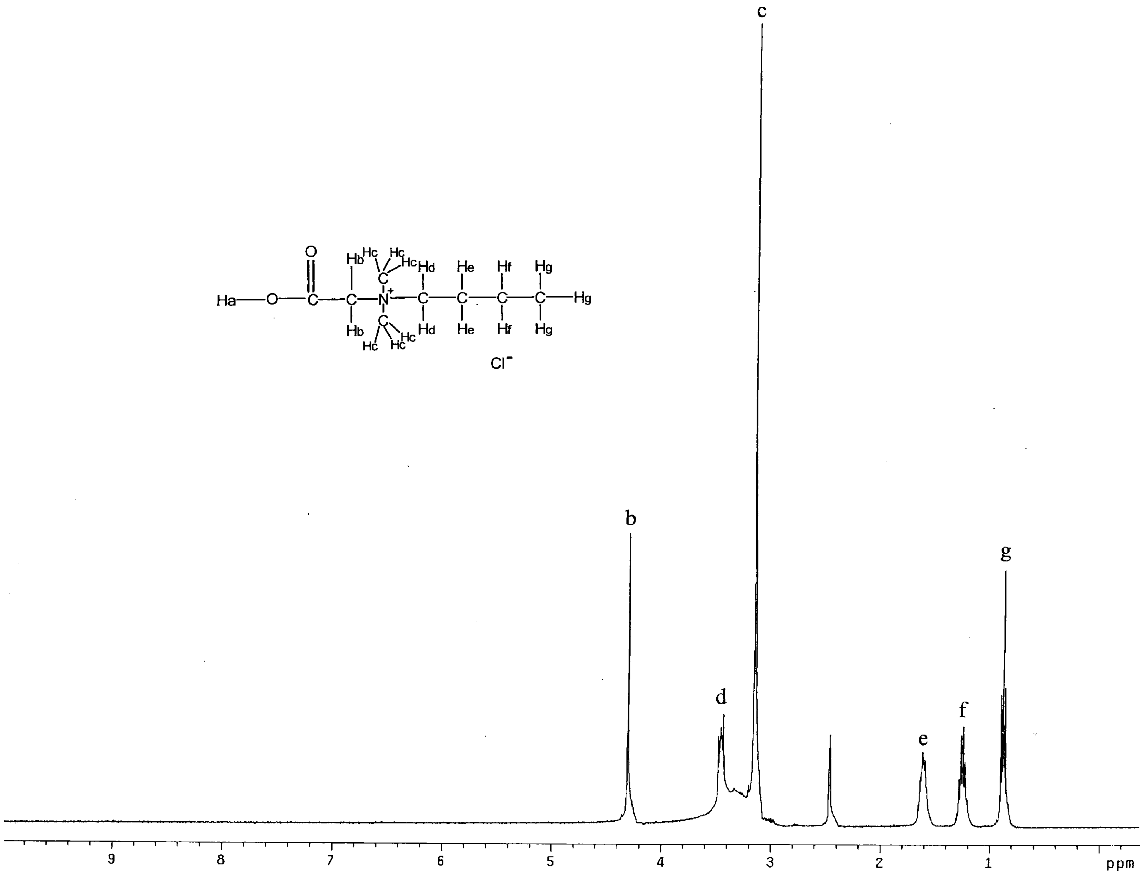


Fig. 6. ^{1}H NMR spectrum of carboxymethyl-butyl-dimethyl ammonium chloride (CBDAC) (solvent: DMSO-d_6).

However, in our study, instead of being stable at the neutral conditions, the dyes showed the highest stabilities at pH 3. Such a surprising result must be associated with the unique structures of the antimicrobial cationic dyes. These dyes possess both hydrophobic and hydrophilic features, similar to surfactants. With the increase of alkyl chain length, the hydrophobicity of dyes is improved. The surfactant feature of these dyes suggests that the dyes may exist similarly to surfactants in aqueous solutions. The dyes could aggregate in water to form micelles by placing the hydrophilic segment at the outer layer and push the hydrophobic alkyl chain into the inner layer. Since the hydrophilic moiety contains a positively charged nitrogen atom, the hydrophilic sections of the aggregates and the outer layer of the micelles possess positive charges. Thus, the aggregates and micelles tend to attract negatively charged species but to repel positive ones in the solutions. In other words, hydroxide ions (OH^-) can be favorably attracted to the aggregates or micelles but hydronium ions (H^+) will be repelled from them. The close interactions between the OH^- and the dyes would lead to hydrolysis, which explains the lower dye stabilities at higher pH conditions. With the decrease of pH values, the concentration of OH^- decreases, while that of H^+ increases. However, at weak acidic conditions, H^+ may not be fully accessible to the amide bonds due to the repulsion between the positively charged hydrophilic end and H^+, so the hydrolysis may not occur and the stability of the dyes maintains until pH reaches 3. With the further decrease of pH values, the concentration of H^+ is further enhanced. At the same time, the concentration gradient may also

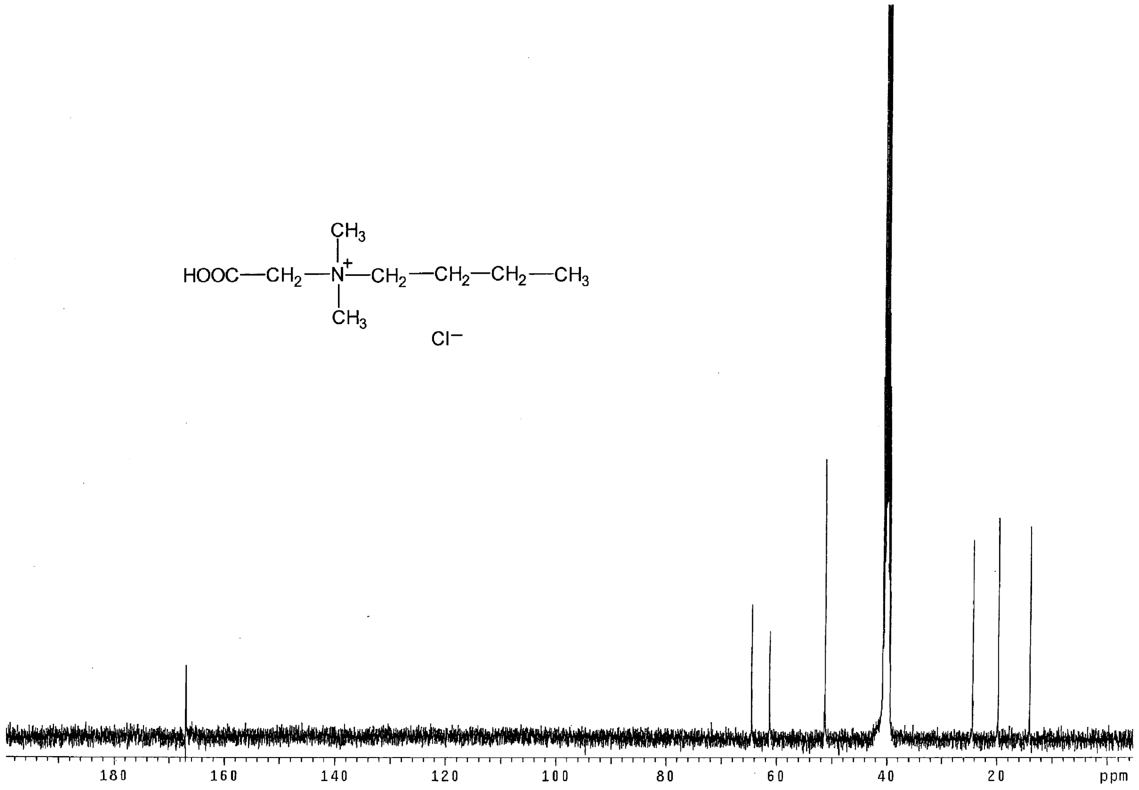


Fig. 7. ^{13}C NMR spectrum of CBDAC (solvent: DMSO-d_6).

increase around the aggregates and micelles, and a strong driving force may thus be formed, surpassing the repulsive interactions. Therefore, H^+ may be able to come close to the aggregates or micelles, the hydrolysis of the amide groups begins to follow the "normal" acid-catalyzed hydrolysis pathway, and the stability of the dyes gradually decreases accordingly.

It should be pointed out that this attraction/repulsion induced hydrolysis of amide linkage is not an unusual phenomenon. In studying the hydrolytic stability of acethydrazide and acetamide, Edward et al. [17] reported in 1955 that the former was hydrolyzed more slowly than the amide in weak acid because the positive charge of the protonated acethydrazide repelled hydrogen ions. In strong acid, however, this effect operated less strongly and the hydrolysis of acethydrazide became fast. Similar results were also reported by Lindgren and Niemann [18], as well as Butterworth and co-workers [19]. All these early results agree well with our findings, indicating that the hypothesis proposed in this study can be a possible mechanism in the hydrolysis of the dyes.

3.4. Effect of alkyl chain length

Different mono-substituted dyes showed different stabilities under various pH conditions, which can also be seen in Fig. 9. For example, under acidic conditions m-4 was more stable than m-8, which, in turn, was more stable than m-12. As mentioned earlier, the hydrolysis of the dyes occurred due to the cleavage of the amide linkages, the hydrolysis products were 1-amino anthraquinone and the corresponding carboxyalkyl ammonium chlorides (Eq. (1)). Carboxyalkyl ammonium

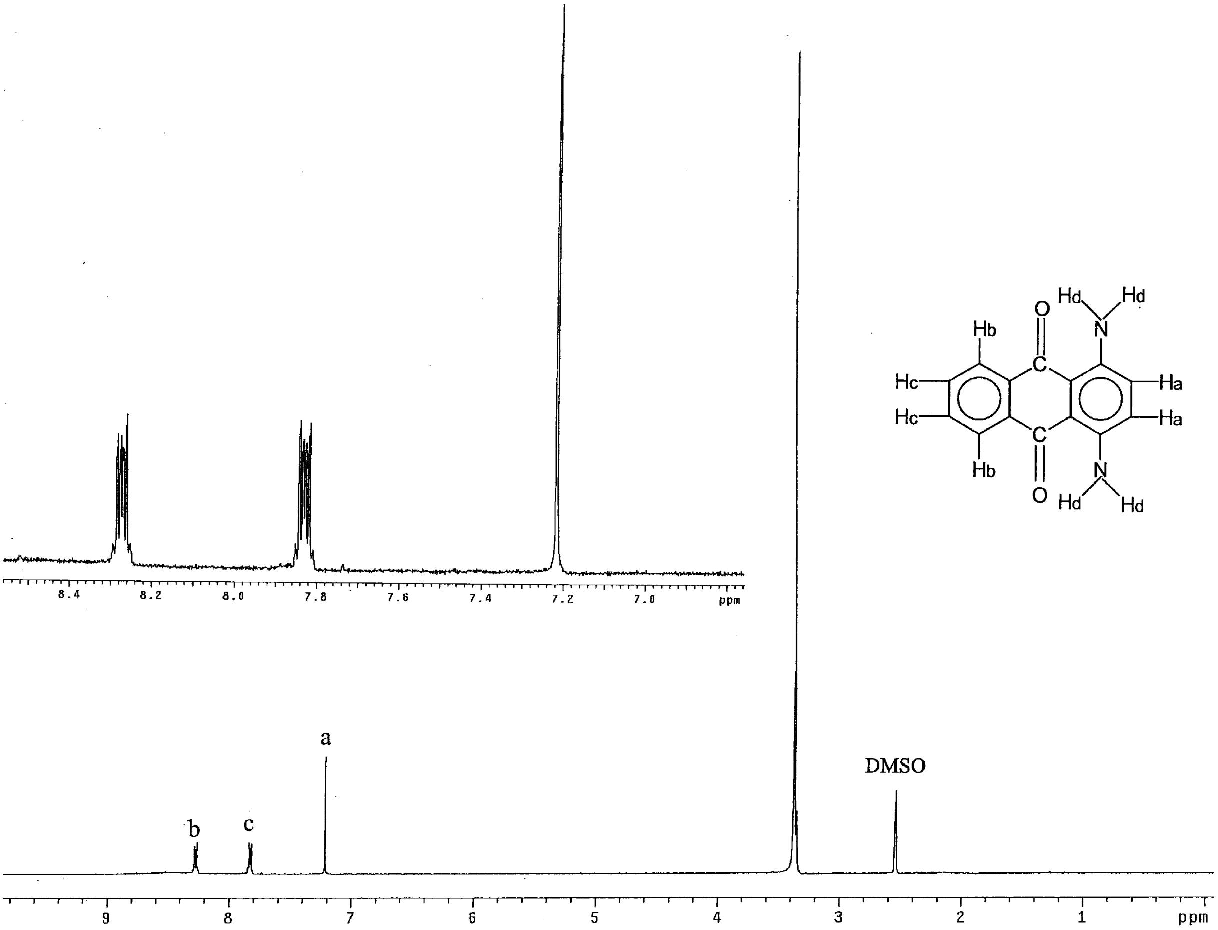


Fig. 8. ^{1}H NMR spectrum of 1,4-diamino anthraquinone (solvent: DMSO-d_6).

chlorides belong to a type of amphoteric surfactants named alkylbetaine [20]. The solubility of alkylbetaines in water was highly dependent on the number of carbon atoms [20]. Usually, the higher alkyl chain length in betaines, the lower the solubilities. For example, ethanesulfobetaine with a hexyl alkyl chain has a solubility of 655.0 g/l at 30 °C, while the solubility of the decyl homologue drops to 2.2 g/l, and that of the docosyl homologue was less than 0.1 g/l [21]. Similarly, in our case, butylbetaine should be more soluble than octylbetaine, which, in turn, must have a higher solubility than dodecylbetaine.

The different solubilities of alkylbetaines may explain the different stabilities of the three monosubstituted dyes. Eq. (1) is an equilibrium existing in the hydrolysis of the dyes. In the case of m-8, the hydrolyzed product octylbetaine is less soluble in water compared to m-4 (butylbetaine), so it may precipitate out, leading to a lower concentration in the hydrolysis solution, and thus drive the hydrolysis equilibrium to the right hand side. Therefore, compared to m-4, lower stability was observed in m-8. The hydrolysis of m-12 produces dodecylbetaine, which is even less soluble than octylbetaine, indicating that the concentration of dodecylbetaine is even lower in the solution and the equilibrium is shifted further to the right hand side. So it is not surprising to observe the lowest stability in m-12. However, when pH is higher than 7, all the alkylbetaines may become deprotonated and the solubilities may be increased

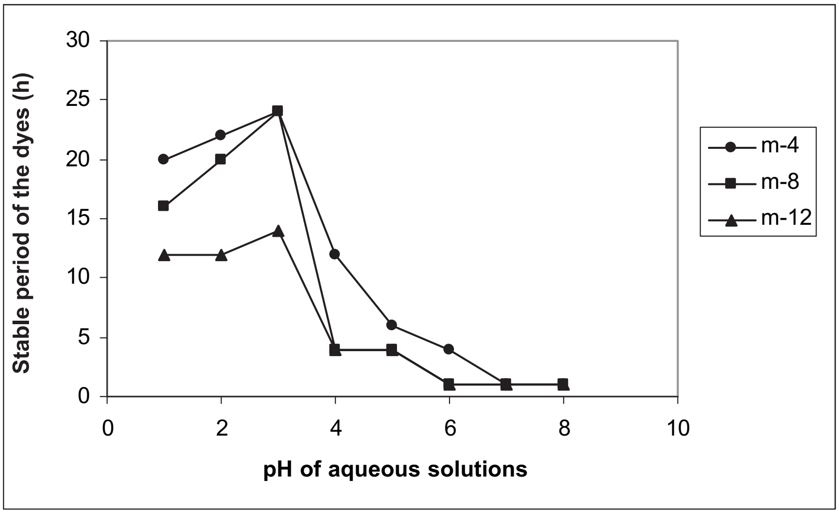


Fig. 9. Stability of mono-substituted dyes at different pH and 100 °C (original dye concentrations: 1 mMol/L).

compared with the protonated forms (Eq. (1)). Therefore, the hydrolysis equilibriums of three dyes may not be affected as much as in acidic conditions, so all the dyes showed similar stabilities at pH higher than 7.

The above explanation was supported by observation of resulted opaque solution when pH was adjusted lower than 3, particularly in the hydrolysis of m-8 and m-12. The opaque solutions were caused by the low solubilities of octylbetaine and dodecylbetaine. In acidic conditions, most of them exist in the protonated form that has relatively low solubility in water. In basic conditions, however, the deprotonated form becomes predominant in the solutions (Eq. (1)). The relatively high solubility of the deprotonated alkylbetaines may decrease the oily droplet formation. In the case of butylbetaine, due to its high solubility in both protonated and deprotonated forms, no opaqueness was observed in the hydrolysis process.

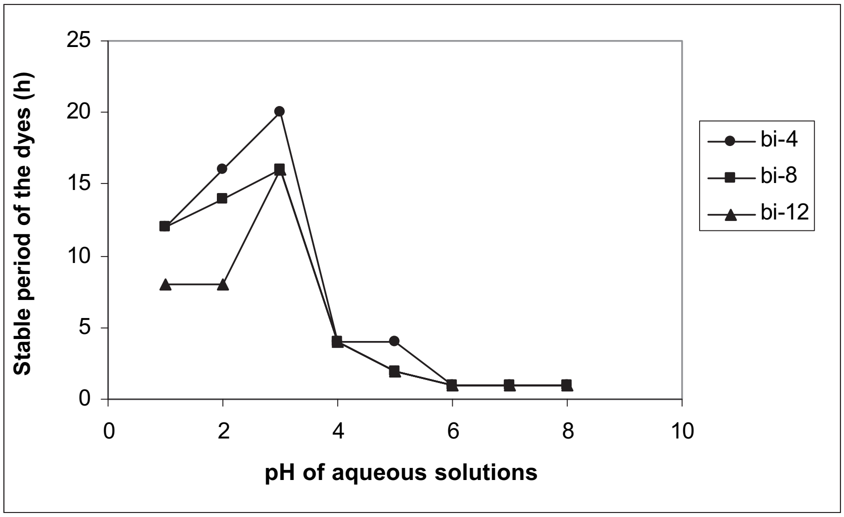


Fig. 10. Stability of bi-substituted dyes at different pH and 100 °C (original dye concentrations: 1 mMol/L).

The effect of pH on the hydrolysis of bi-substituted dyes was also investigated, and the results are shown in Fig. 10. Generally speaking, both the mono- and bi-substituted dyes exhibited a similar stability trend with respect to pH, but the bi-substituted ones had an overall lower stability. We believe this may be due to the fact that the bi-substituted dyes have two amide linkages in each compound, which make them more susceptible to acid or base attack compared with the mono-substituted dyes. Therefore, lower stability was observed in the bi-substituted dyes. To this point, all the results indicate that the two series of dyes are stable within the range of pH 1–4 at 100 °C. As mentioned earlier, in acrylic dyeing applications, the dyes may be exposed to acidic conditions at high temperatures. We are confident that these new antimicrobial cationic dyes can withstand all the application conditions encountered in cationic dyeing processes.

4. Conclusions

The thermal and hydrolytic stabilities of the antimicrobial cationic dyes were investigated. Both mono-substituted and the bi-substituted cationic dyes were stable below 190 °C. Above 200 °C, these dyes may decompose in two steps, the first one of which was the decomposition of QAS components, while the second step was due to the decomposition of the anthraquinone structures. These dyes were more stable in acidic solutions than in basic conditions. The dyes showed highest stability at pH 3. The hydrolysis of the dyes occurred at the amide bond between the anthraquinone and quaternary ammonium salt groups. The increase of alkyl chain lengths in the QAS component caused decreased stability to the dyes.

Acknowledgements

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