Determination of melamine derivatives, melame, meleme, ammeline and ammelide by high-performance cation-exchange chromatography

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Abstract

The thermal decomposition of melamine produces melame and meleme, while alkaline hydrolysis of melamine produces ammeline and ammelide. These four melamine derivatives were determined by high-performance cation-exchange chromatography using phosphate buffer as the eluent. The elution behavior during the isocratic and gradient elutions was examined and the determination was simultaneously achieved using photodiode array UV–Vis detection. An isocratic elution with 50 mM phosphate buffer (pH 2.5) seemed most suitable for the rapid and quantitative analysis. Three types of gradient elutions involving phosphate- and NaCl-concentrations, and pH also showed satisfactory separations for the melamine derivatives.   © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Melamine has been used in the production of melamine–formaldehyde resins for surface coatings, laminates, and adhesives and in the production of flame retardants. Melamine is produced by the thermal condensation of dicyandiamide at over 300°C. During this process, some de-ammonia condensation derivatives, including melame and meleme, are produced as shown in Fig. 1 [1,2]. These melamine derivatives have already been reported since 1834 [3–5]. However, there have been

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few studies on their physical and chemical properties because these melamine derivatives are chemically inert but show remarkably low solubility in aqueous solutions and organic solvents. Takimoto et al. [6,7] studied the synthesis, isolation of melame and meleme, and their characterization based on their IR and UV absorption spectra about 30 years ago. These derivatives could be separated using cation-exchange chromatography (Amberlite IR-112, 20×0.8 cm I.D., 0.5 M hydrochloric acid). However, the elution of melame and meleme in this system took 9–17 h and their determination was achieved only after isolation. Thus, their rapid and quantitative analysis has not been done satisfactorily. Due to these analytical
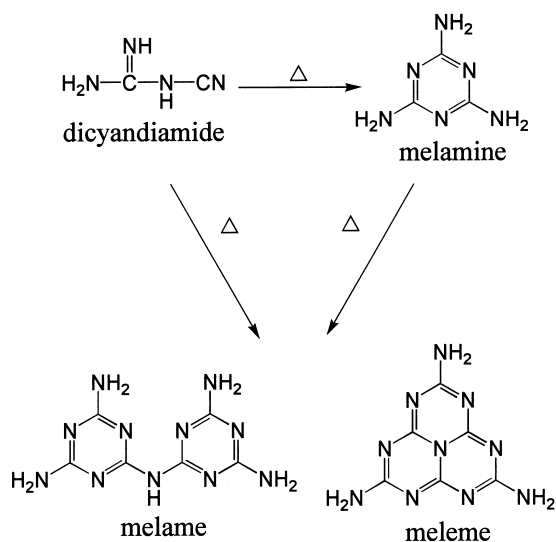
Fig. 1. Formation of melame, melamine and meleme by the thermal decomposition of dicyandiamide.

problems, the fundamental study and the application of these derivatives are still unexplored.

Recently, we reported a method for the rapid and quantitative determination of meleme using high-performance cation-exchange chromatography (HPCEC) with 20 mM phosphate buffer as the eluent [8]. The use of UV–Vis photodiode array detection made the simultaneous separation and determination of meleme possible. The phosphate buffer at an appropriate pH gave good separation because the elution behavior of meleme depends on the pH of the eluent. However, melame could not be analyzed satisfactorily in the HPCEC system under the same conditions. Since melame was eluted more slowly than melamine and meleme in Takimoto's system [6], melame was probably strongly adsorbed on the stationary phase, and hence was undetectable in our previous HPCEC system. Thus, no satisfactory sepa-

ration of melame using liquid chromatography has been reported so far.

In this paper, the melamine thermal decomposition derivatives, melame and meleme, were determined for various combinations of concentrations (50–500 mM) and pH of the eluent phosphate buffer during isocratic elution. Melame was satisfactorily separated from melamine and meleme depending on both the concentration and pH. Moreover, gradient elutions based on phosphate- and NaCl-concentrations, and pH led to their good separation. In addition, the alkaline hydrolysis of melamine gives mainly two products, i.e., ammeline and ammelide [9] as shown in Fig. 2. These derivatives are also produced during the production of melamine as by-products and their HPCEC analysis has been reported by Debowski and Wilde [10]. Our isocratic and gradient elution systems have also achieved a rapid and quantitative separation. Finally, the analysis of microamount impurities in commercially available melamines for industrial use was tested using our system, demonstrating the usefulness of our rapid HPCEC system for analysis of melamine derivatives such as melame, meleme, ammeline and ammelide.

2. Experimental

2.1. Chemicals

Phosphoric acid and sodium hydroxide were of analytical-reagent grade (Wako). Water was distilled and filtered through an ultrapure water system (Advantec). The purified melamine, meleme and cyanuric acid were kindly supplied by Nissan Kagaku Kogyo. Dicyandiamide was purchased from Sigma. All other reagents were obtained from commercial sources and used without further purification. Melame was synthesized by thermal condensation of
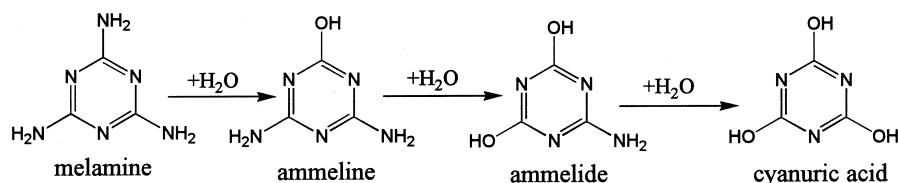

Fig. 2. Formation of ammeline, ammelide and cyanuric acid by the alkaline hydrolysis of melamine.

dicyandiamide according to the method of Takimoto et al. [6]. The crude melame was purified by recrystallization. According to the method of Takimoto et al. [9], ammeline and ammelide were isolated from the product mixture by the alkaline hydrolysis of melamine. All melamine derivatives were established by elemental analysis, electron impact mass spectrometry, infrared absorption spectroscopy, and solid-state ^{13}C-NMR.

2.2. Equipment

HPCEC analyses were carried out using a Shimadzu LC-6AD high-performance liquid chromatograph equipped with a photodiode array spectrometric detector (SPD-M6A), system controller (SCL-6B), column oven (CTO-6A), and SPD-6A data analysis system (M6PAC ver. 2.1). A Partisil-10 SCX analytical column (10 mm particle size, 250× 4.6 mm I.D., GL Sciences) and guard column (10× 4.0 mm I.D., GL Sciences) of the same material were used.

2.3. Preparation and procedure

First of all, to determine the correct concentrations of the melamine derivatives for quantitative analysis, their solubilities were initially examined using the following procedure. Small amounts (10–30 mg) of melame, meleme, ammeline, and ammelide were individually added to 1 l of water and stirred for 4 h at 60°C. The solutions were filtered through a 0.5 μm membrane filter (Advantec) to remove any insoluble material. From the comparison of the weights of the membrane filters after and before the filtration, the solubilities of melame, meleme, ammeline and ammelide were approximated, and then their stock solutions with concentrations of 36, 11, 160, and 170 μM were used for the quantitative analyses, respectively. Since other compounds, melamine, dicyandiamide and cyanuric acid, show relatively higher solubilities, 100, 150 and 500 μM stock solutions were prepared, respectively.

2.3.1. Isocratic elution

The samples for the evaluation of the separation were prepared according to the following procedure. Each melamine derivative was individually dissolved

in water to prepare the stock solutions at the concentrations previously described. After stirring for 4 h at 60°C, the solutions were filtered through the membrane filter. Equal amounts of these stock solutions of melame, melamine, meleme and dicyandiamide were combined for the evaluation of their separation. The other sample, containing ammelide, ammeline, melamine and cyanuric acid, was also similarly prepared. The concentrations of these derivatives in the sample solutions were 9.0 μM (melame), 2.8 μM (meleme), 25 μM (melamine), 40 μM (ammeline), 43 μM (ammelide), 38 μM (dicyandiamide), and 125 μM (cyanuric acid).

Phosphate buffers (50–500 mM) at various pH values between 2.0–6.0 were prepared as eluents for the isocratic elution. The flow-rate was 0.5 ml min^{-1} and the oven temperature was 40°C. A 20 μl aliquot of each sample solution prepared above was injected, and the elution was monitored at 223 nm, except for cyanuric acid, and scanned between 200 and 300 nm using the photodiode array detector. The elution of cyanuric acid was monitored at 215 nm. Linear calibration lines for all derivatives were obtained.

In order to apply our HPCEC system to an analysis of the impurities in melamine for industrial use, four commercially available melamines (200 mg) were dissolved in 1 l of water. All of the melamine samples including their impurities were completely soluble under these conditions. After stirring for 4 h at 60°C, the solutions were filtered through the membrane filter. The impurity contents were estimated by their linear calibration lines.

2.3.2. Gradient elutions

On the basis of the isocratic elution results, these conditions (50 mM phosphate concentration, pH 2.5) were used for the gradient elutions. The sample solutions were prepared according to the following procedure. Small amounts of melamine, melame, meleme and dicyandiamide were dissolved in water and the solution was stirred for 4 h at 60°C followed by filtration using the membrane filter. The concentrations for the melamine thermal decomposition derivatives were 7.9 μM (melamine), 1.3 μM (melame), 1.4 μM (meleme) and 12 μM (dicyandiamide). The other sample solution for the melamine alkaline hydrolysis derivatives was also prepared in the same way and the concentrations for

melamine, ammeline, ammlelide and cyanuric acid were 7.9, 6.3, 7.0 and 16 μM, respectively. The elutions were monitored at 223 and 215 nm for the thermal decomposition and alkaline hydrolysis derivatives, respectively, and scanned between 200 and 300 nm using the photodiode array detector.

3. Results and discussion

The elution behavior of a mixture of melame, melamine and meleme was examined using 50–500 mM phosphate buffer as the eluent at various pH values (pH 2.0–6.0). Dicyandiamide was added to this mixture for comparison of the elution behavior with those of these derivatives because this is a starting material for the production of melamine. Fig. 3 shows the pH-dependent elution profiles of these four compounds using 50 mM phosphate buffer. In the range of pH 2.0–4.0, all four tested compounds were satisfactorily separated. Each peak was identified by comparison with their standard UV spectra using the photodiode array detection. At pH 2.0, melame (peak 4 in Fig. 3) was the most slowly eluted and showed a broad peak. The UV spectrum measured at the tailing points suggested that any tailing might not result from impurities (data not
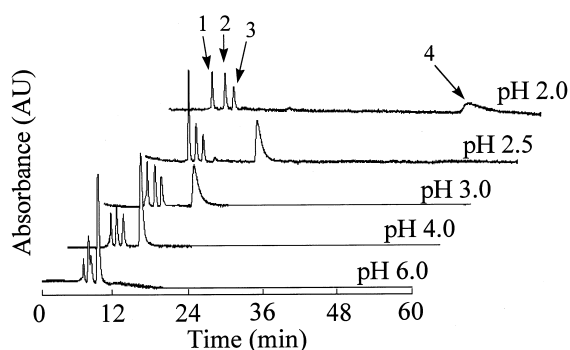
shown). With increasing pH, melame was eluted faster and the peak tailing gradually decreased. The peak heights appeared to change depending on the pH of the eluent phosphate buffer because the UV spectra of the derivatives also changed depending on the pH. In addition, linear calibration lines were obtained for melame, melamine and meleme within the ranges of 9.0–36.2 μM, 11.9–85.5 μM and 2.8–10.6 μM, respectively. The correlation coefficients were 0.9992, 0.9998 and 0.9994, respectively (data not shown).

Fig. 4 shows the pH-dependent elution behavior of ammeline, ammelide and melamine (peaks 1, 2 and 3, respectively) using 50 mM phosphate buffer. Since cyanuric acid is produced during the production of melamine from urea, it was also analyzed under the same conditions by detection at 215 nm. Cyanuric acid was found to elute faster than ammelide (see Fig. 5), but its peak is not shown in Fig. 4 because it was undetectable at 223 nm. Although the elution behavior of these derivatives except for ammeline was not influenced by the pH and concentration of eluent phosphate buffer, these three compounds, ammeline, ammelide and melamine, were satisfactorily separated in the range of pH 2.0–6.0. Linear calibration lines were also obtained
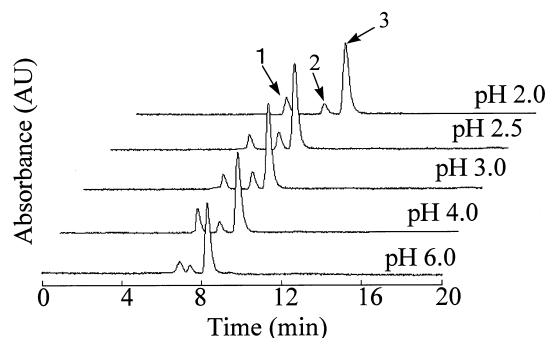

Fig. 3. pH-Dependent elution profiles of melame (4), melamine (3), meleme (2) and dicyandiamide (1) during isocratic HPCEC using 50 mM phosphate buffer in the pH range of 2.0–6.0. The flow-rate was 0.5 ml min^{-1} and the oven temperature was 40°C. A 20 μl aliquot of the sample solution was injected and the elution was scanned between 200 and 300 nm. The elution profiles are expressed by monitoring at 223 nm. The sample preparation was described in the experimental section, and the concentrations of melame, meleme, melamine and dicyandiamide were 9.0, 2.8, 25, and 38 μM, respectively.

Fig. 4. pH-Dependent elution profiles of ammeline (1), ammelide (2) and melamine (3) during isocratic HPCEC using 50 mM phosphate buffer in the pH range of 2.0–6.0. The flow-rate was 0.5 ml min^{-1} and the oven temperature was 40°C. A 20 μl aliquot of the sample solution was injected and the elution was scanned between 200 and 300 nm. The elution profiles are expressed by monitoring at 223 nm. This analyzed sample also contained cyanuric acid that was detectable at 215 nm but not at 223 nm. The sample preparation was described in the experimental section, and the concentrations of ammeline, ammelide, melamine and cyanuric acid were 40, 43, 25 and 125 μM, respectively.
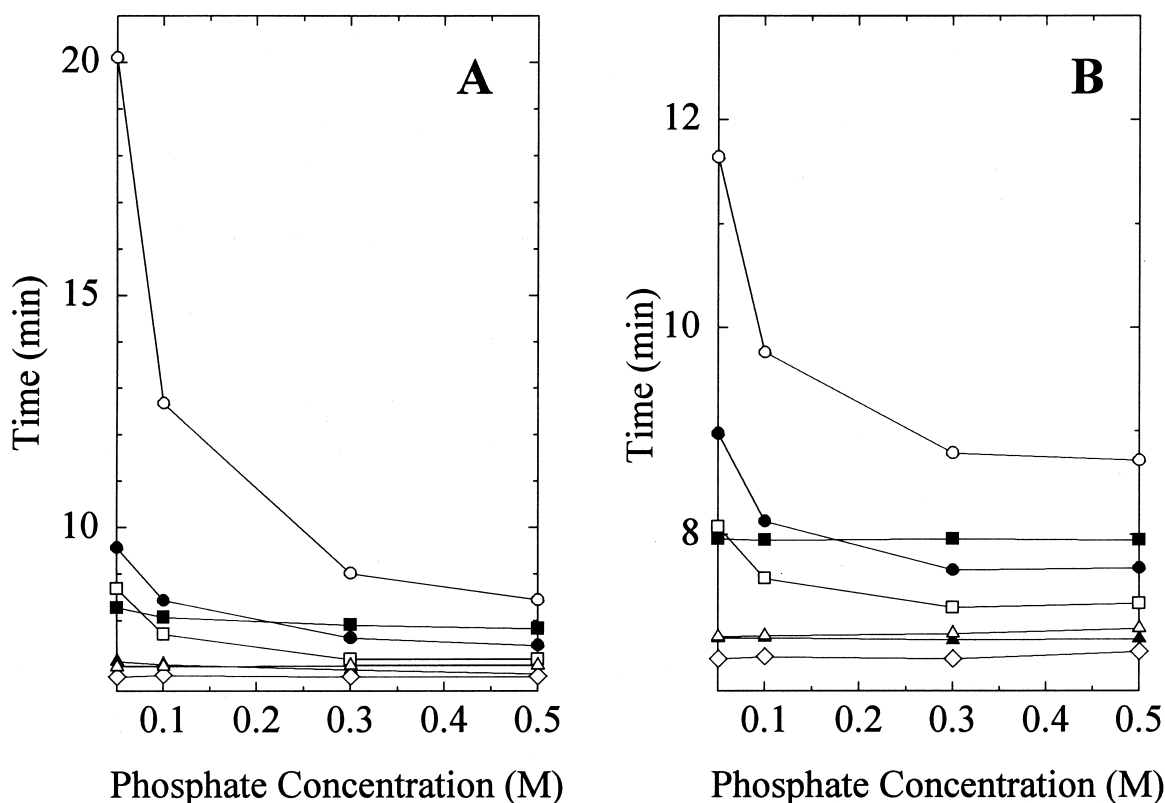
Fig. 5. Phosphate concentration dependent change in elution times of melamine derivatives and the raw materials at pH values of 2.5 (A) and 4.0 (B). Elution times are given as means for triplicate experiments. Symbols: ammeline (□), ammelide (▲), cyanuric acid (◇), dicyandiamide (△), melame (○), melamine (●) and meleme (■). The elution conditions were the same as described in Fig. 3 and Fig. 4.

for ammeline and ammelide within the ranges of 8.3–146 μM and 9.8–85.7 μM, respectively. The correlation coefficients were 0.9996 and 0.9994, respectively (data not shown).

Effects of the phosphate concentration on the elution times of these melamine derivatives and their starting materials were compared between pH values 2.5 and 4.0 as shown in Fig. 5A and B, respectively. The elution time of melame drastically changed depending on the phosphate concentration (50–300 mM) at pH values of both 2.5 and 4.0. Melame was eluted faster with increasing concentrations of the phosphate buffers. Similar small changes in the elution times were also observed for melamine and ammeline under the same conditions. However, there was no change in the elution times of the other compounds, ammelide, cyanuric acid, dicyandiamide and meleme. In contrast, increasing the phosphate

concentration (100 to 300 mM) reversed the order of elution between melamine and meleme at both pH values. These results indicate that the elution behavior of these melamine derivatives is not influenced in the same way by the pH and concentration of the phosphate buffer.

To examine the availability of salt-gradient elution, gradient elutions using phosphate- and NaCl-concentrations were conducted. Fig. 6 shows the phosphate-concentration gradient elution profiles at pH 2.5 for the seven melamine derivatives. The thermal decomposition derivatives (melame, melamine, meleme, and dicyandiamide) and alkaline hydrolysis derivatives (ammeline, ammelide, cyanuric acid, and melamine) were satisfactorily separated as shown in Fig. 6A and B, respectively. In the case of these gradient elutions, each UV spectrum of the peak top is shown in Fig. 7. There was no
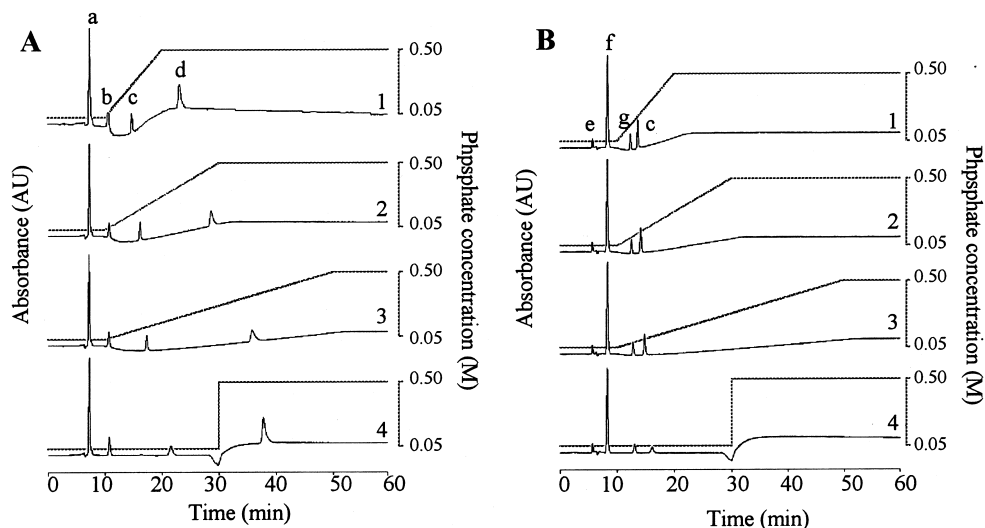
Fig. 6. Effect of the phosphate-concentration gradients on the elutions of the melamine thermal decomposition (A) and alkaline hydrolysis derivatives (B). Four kinds of phosphate-concentration gradient elutions (0.05–0.5 M) were conducted using 10 mM and 1 M phosphate buffers at pH 2.5. Peaks: dicyandiamide (a), meleme (b), melamine (c), melame (d), cyanuric acid (e), ammelide (f) and ammeline (g). The flow-rate was 0.5 ml min^{-1} and the oven temperature was 40°C. A 20 μl aliquot of each sample solution was injected and the elution was scanned between 200 and 300 nm. The elution profiles for the thermal decomposition and alkaline hydrolysis derivatives are expressed by monitoring at 223 and 215 nm, respectively. The sample preparation was described in the experimental section, and the concentrations of melamine, melame, meleme, dicyandiamide, ammeline, ammelide and cyanuric acid were 7.9, 1.3, 1.4, 12, 6.3, 7.0 and 16 μM, respectively.

significant difference in the spectra between the phosphate-concentration gradient and isocratic elutions. In addition, the NaCl-concentration gradient
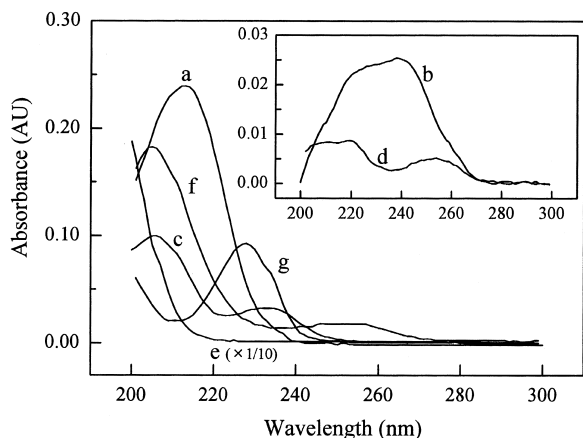

Fig. 7. UV spectra for the seven melamine derivatives, dicyandiamide (a), meleme (b), melamine (c), melame (d), cyanuric acid (e), ammelide (f) and ammeline (g). These spectra are expressed as the snapshots at their peak tops in the gradient 1 pairs in Fig. 6A and B.

elution in 50 mM phosphate buffer at pH 2.5 also gave good separation as shown in Fig. 8. No significant spectral change was observed under these gradient conditions. In these phosphate- and NaCl-concentration gradient elutions, the dissociation states of the melamine derivatives were unchanged but increasing ionic strength of the eluent led to reducing the adsorption of the melamine derivatives, especially melame, on the cationic-exchange solid support.

A pH-gradient elution showed good separation for the melamine derivatives using 50 mM phosphate buffer as shown in Fig. 9. Under these conditions, these seven derivatives gave different elution times from each other, suggesting that a mixture with these thermally decomposed and alkaline hydrolysis products can be simultaneously analyzed using this pH-gradient elution. Although some pH-dependent changes in the absorption spectra of melame, meleme and ammeline were observed due to the changes in the dissociation states [11], the determination could be easily done using the photodiode array detection. However, melame was eluted very
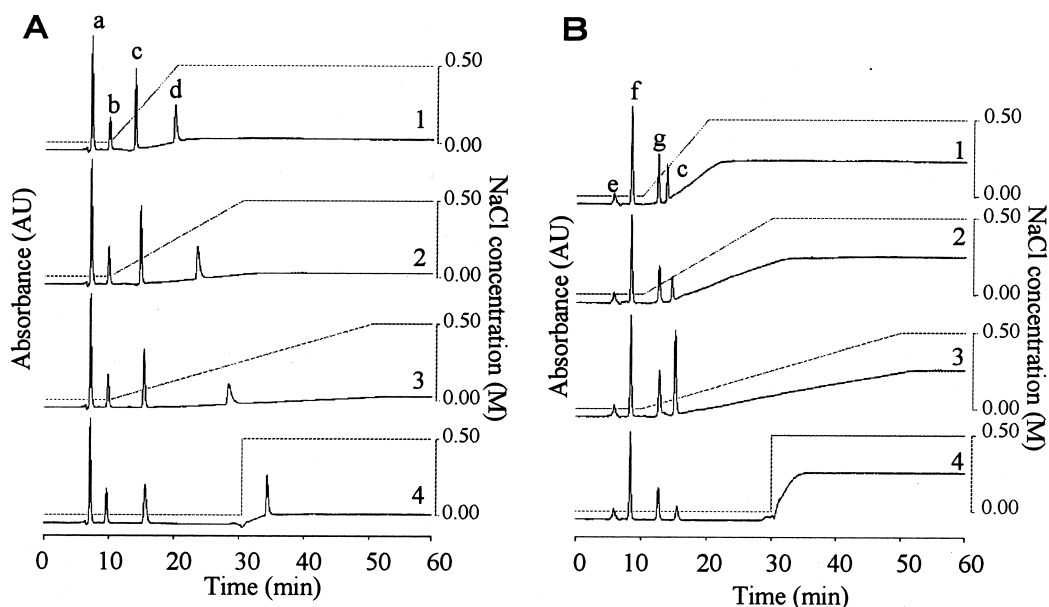
Fig. 8. Effect of the NaCl-concentration gradients on the elutions of the melamine thermal decomposition (A) and alkaline hydrolysis derivatives (B). Four kinds of NaCl-concentration gradient elutions (0.05–0.5 M) were conducted using 50 mM phosphate buffers with 1 M and without NaCl. All other analytical conditions were the same as those described in Fig. 6.

slowly with an elution time of 64 min. Changing the conditions is required for a rapid analysis.

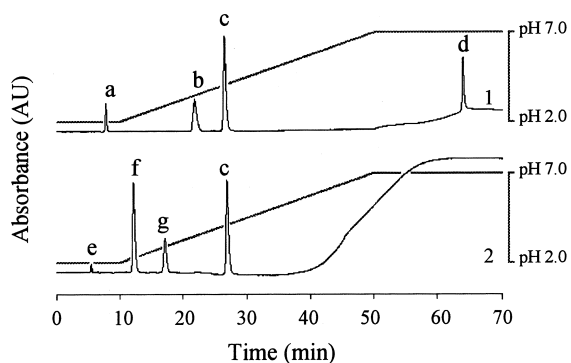For the quantitative analysis of the melamine

Fig. 9. Effect of the pH-gradients on the elutions of the melamine thermal decomposition (upper) and alkaline hydrolysis derivatives (lower). The elution pH was raised from 2.0 to 7.0 using 50 mM phosphate buffers at pH 1.8 and 9.2. Peaks: dicyandiamide (a), meleme (b), melamine (c), melame (d), cyanuric acid (e), ammelide (f) and ammeline (g). The elution profiles for the thermal decompostion and alkaline hydrolysis derivatives are expressed by monitoring at 225 and 220 nm, respectively. All other analytical conditions were the same as those described in Fig. 6.

derivatives, the isocratic elution seems more suitable than the other three types of gradient elutions because a fluctuation in the base-line induced by the three types of gradient elutions caused poor correlation coefficients. A good correlation between the amounts of the injected samples and their peak areas is required for the quantitative analyses of the samples with low solubilities. Moreover, during the analysis of the melamine derivatives, the time (24 min) required for one cycle of the analysis in the isocratic elution was shorter than those in the phosphate- and NaCl-concentration gradient elutions (at least 30 min) and in the pH-gradient elution (over 70 min). The isocratic elution was also advantageous from this point of view. As a result of this work, the isocratic elution using 50 mM phosphate buffer (pH 2.5) was recommended for the quantitative analysis of melamine derivatives as the optimum conditions.

Finally, using our HPCEC isocratic system, we analyzed the impurities in four commercially available melamines for industrial use. Fig. 10 shows their HPCEC profiles with 50 mM phosphate buffer (pH 2.5) as the eluent. The impurities were estimated as shown in Table 1 in which the contents can be
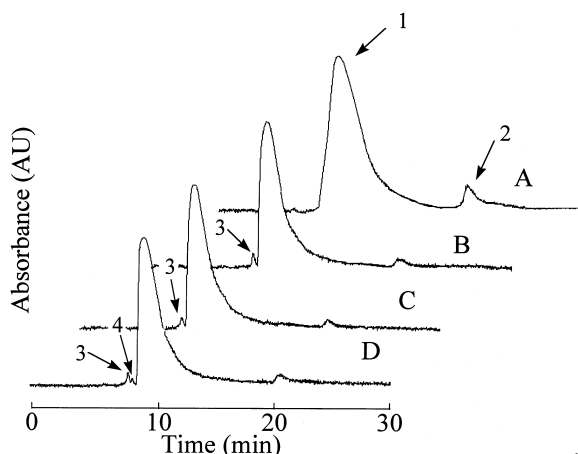
Fig. 10. HPCEC profiles of four commercially available melamines with 50 mM phosphate buffer (pH 2.5). Peaks: melamine (1), melame (2), ammeline (3) and meleme (4). A 20 µl aliquot of each melamine solution containing impurities was injected. The sample preparation was described in the experimental section and all other analytical conditions were the same as those described in Fig. 3.

Table 1
Impurity contents in the four commercially available melamines estimated using the isocratic HPCEC with 50 mM phosphate buffer (pH 2.5)

Sample	Impurity	Content(%)	
		This method[a]	Other method[b]
A	melame	0.20	0.15
B	melame	0.08	0.05
	ammeline	0.15	0.05
	ammelide	n.d.[c]	0.04
	ureidomelamine[d]	n.d.	0.15
C	melame	0.07	0.05
	ammeline	0.12	0.05
	ammelide	n.d.	0.04
	ureidomelamine	n.d.	0.15
D	meleme	0.05	n.d.
	melame	0.10	n.d.
	ammeline	0.10	0.03
	ammelide	n.d.	0.05
	ureidomelamine	n.d.	0.14

[a] The impurity contents were estimated by their linear calibration lines.
[b] The detailed method is not shown because of an industrial requirement.
[c] n.d.: not detected.
[d] $NH_2CONH–C_3H_4N_3$.

compared to those determined by other methods (the details are not shown due to an industrial requirement). Melame, meleme and ammeline were determined as impurities (samples A, B, C and D), but not ammelide (samples B and C) because its peak must overlap with the major peak of melamine. Ureidomelamine was undetectable in our system (samples B, C and D) and an improvement in the analytical conditions is in progress. As a result of this analysis, it was demonstrated that our HPCEC system gives a rapid qualitative and quantitative analysis of melamine derivatives although more improved conditions should be required.

In conclusion, melamine derivatives such as melame, meleme, ammeline and ammelide were rapidly separated and satisfactorily determined based on pH and concentration of the eluent using our HPCEC system. Particularly, the use of photodiode array UV–Vis detection made the identification and the purity estimation for these derivatives quite easy, even if their suitable wavelengths for detection were unknown or different from each other. Our HPCEC system should be suitable for studies on the reactivity and stability of even less-known compounds such as melame and meleme.

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