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Notes

A Multiple Processing Hybrid Flow System for Analysis of Formaldehyde Contamination in Food

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This work proposes a flow system suitable for the rapid screening of formaldehyde contaminated in food. The system is based on the concept of a flow analyzer with a Hantzsch reaction. An operating procedure was developed for multiple tasking and high sample throughput. This resulted in a significant sample throughput of 51 samples h^{-1}. Under the optimized conditions, linear calibration from 10 to 100 μM was obtained. The system gave a limit of detection and a limit of quantitation of 0.06 and 0.10 mg kg^{-1}, respectively. The system was successfully applied to re-hydrated dry squids, vegetables and mushrooms.

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Introduction

Formaldehyde (HCHO) is a human carcinogen known to increase mortality from nasopharyngeal cancer.[1] According to the US EPA,[2] the reference dose for chronic exposure (RfD) of HCHO is 0.2 mg kg^{-1} body weight per day. However, the enzymatic reduction of trimethylamine oxide in fish could produce HCHO as a product at levels of up to 98 mg kg^{-1}, causing an increase in the amount of HCHO during the storage of frozen fish.[3,4] In some countries, HCHO is illegally added to foods as a preservative.[5-8] It is therefore necessary to have a method for the detection and monitoring of the level of HCHO in food.

Conventional colorimetric methods[7,9,10] for the qualitative and quantitative analyses of HCHO in food are laborious. A pulsed amperometric method[6] and an "electronic nose"[11] have been reported as alternatives to the conventional methods for the detection of HCHO abuse in seafood. A review of methods for the determination of HCHO in the diet has recently been published.[12] In our view, the most practical and effective technique for detection of the HCHO in food is a method reported by Nash.[13] This method is based on a reaction between HCHO and a diketone in the presence of ammonium acetate. The reaction is also known as the Hantzsch reaction.

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In flow injection analysis (FIA), the Hantzsch reaction has been applied to the analysis of air,[14–17] breath,[18] beverage[19,20] and food.[21] Different diketones, such as 2,4-pentanedione,[16,17,20] 1,3-cyclohexadione[14,21] and 5,5-dimethylcyclohexane-1,3-dione[15,18] have been employed. Amongst these, 2,4-pentanedione is recommended as the best reagent due to its reactivity at lower temperature and low sensitivity to interferences.[16] In a recent flow system, 2,4-pentanedione and ammonium acetate solutions were arranged to mix together in one of the syringes prior to a reaction with HCHO.[17] This flow system for the analysis of HCHO in air is based on the concept of a hybrid flow analyzer.[22] The system is more robust than the conventional FI system due to using syringe pumps.

In this work, a flow system based on hybrid flow[22] was developed for the analysis of HCHO in food. The procedure is different from one presented earlier by Eom *et al*. for monitoring HCHO in air.[17] A new operating procedure has been designed that allows for the processing of 3 samples at one time to achieve a considerably improvement in the sample throughput.

Experimental

Reagents and chemicals

All chemicals used were of analytical reagent grade. Deionized-distilled water used throughout the work was obtained from an EASYpure II system (Branstead, USA).

Ammonium acetate solution (2.0 M) was prepared by

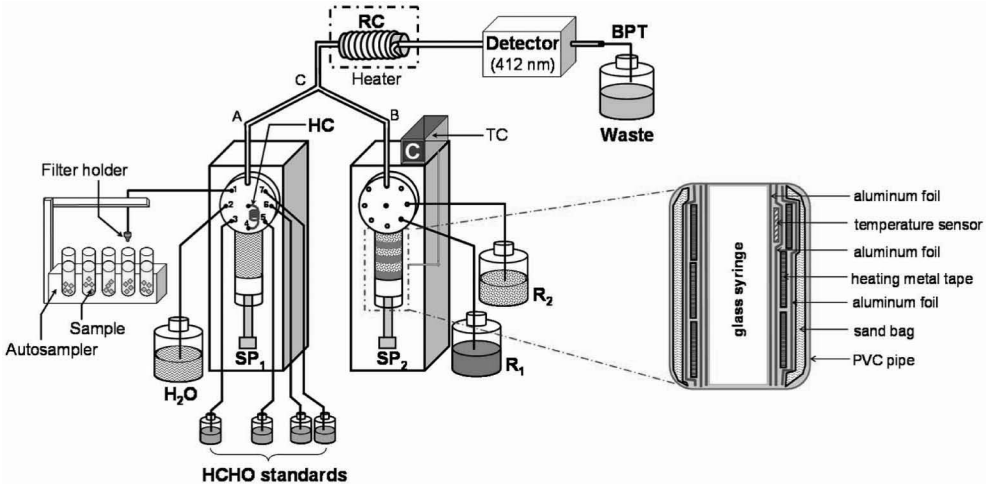


Fig. 1 Flow system developed for the detection of formaldehyde contamination in food: SP_1 and SP_2, syringe pumps providing a flow rate of 0.6 ml min^{-1}; HC, holding coil (1 ml); TC, temperature controller; RC, reaction coil (0.8 mm i.d. × 150 cm length); BPT, back pressure tube (0.5 mm i.d. × 15 cm length); R_1, 0.04 M 2,4-pentanedione; R_2, 2.0 M ammonium acetate. The temperature of syringe SP_2 and the reaction coil RC were always set at the same value.

dissolving 38.5 g of ammonium acetate (Ajax, Australia) in 200 ml of water, followed by adjusting the pH to the range of 5.6 to 6.2 using glacial acetic acid (J. T. Baker, USA), and then diluted to 250 ml with water. A 2,4-pentanedione stock solution (1.0 M) was prepared by diluting 5.0 ml of 95% 2,4-pentanedione (Carlo Erba, Australia) to 50 ml with water. A standardized[23] stock solution of HCHO (0.1 M) was prepared by diluting 0.8 ml of 37.5% HCHO (Ajax, Australia) to 100 ml with water, and was stored in a refrigerator. HCHO calibrators were prepared daily by appropriate dilution with water from the stock reagent.

Sample preparation

A food sample was cut into small pieces (4 to 5 mm), accurately weighed (12.5 g) and placed into a 50-ml centrifuge tube containing 25.00 ml of water, the extraction solvent. The extraction of HCHO from food sample was adopted from a method of Wang *et al*.[5] Sample tubes were capped and shaken at 240 rpm for 15 min (IKA Labortechnik, Germany) to extract the HCHO. The sample tubes were then loaded onto the rack of an autosampler, as shown in Fig. 1.

The flow system

The flow system in Fig. 1 consisted of two syringe pumps (Kloehn V6 Pump, USA), each equipped with an eight-port selection valve (Kloehn 17620, USA). Two glass syringes with a 5-ml zero dead volume (Kloehn, USA) were fitted to the pumps. A spectrometer (Jenway Model 6300, UK), with a 5-cm light-path quartz flow cell was used for monitoring absorbance at 412 nm. The flow system was coupled to an autosampler (PerkinElmer AS90, USA). The control of the syringe pumps and data acquisition were carried out using in-house software written using LabVIEW 8.0.™

A sample extract (350 µl) was introduced into the flow system by first drawing the supernatant from the autosampler (*via* port 1) into the holding coil (HC in Fig. 1), and then driving the sample extract to merge with the mixed reagents at point C. Similarly to the introduction of a sample, the HCHO standards were individually loaded into the HC coil (*via* ports 3, 5, 6 and 7).

For the system shown in Fig. 1, filtration of the sample was done on-line *via* a filter holder (Millipore-Swinnex, USA) fitted at the end of a sampling probe and a glass fiber filter (13 mm diameter) placed inside this holder. Small debris of sample extracts was filtered out to prevent clogging of the flow system.

Syringe heater

It is known that heating can improve the sensitivity of the Hantzsch reaction,[21,24] so an electrical heating jacket was constructed for the glass syringe SP_2. This syringe SP_2 pre-heated a mixture of reagents R_1 and R_2 stored in the syringe. The inset of Fig. 1 shows the assembly of the heating apparatus. A temperature controller (TC in Fig. 1) was used to control the temperature. The dashed line around syringe SP_2 indicates the region of the thermostated heating jacket.

Results and Discussion

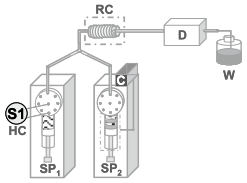
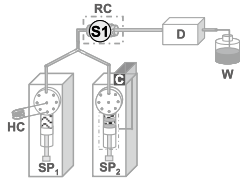
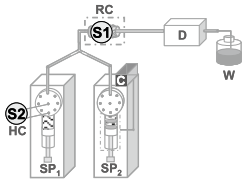
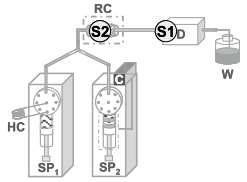
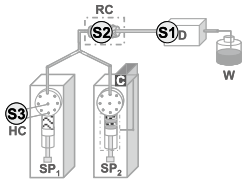
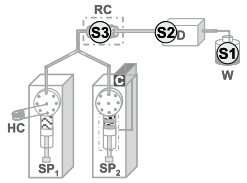
Efficiency of the electrical heating jacket

In order to test the effectiveness of the heating device, the flow system in Fig. 1 was used with and without operating the heating device. As expected, the sensitivity of the system using the heated jacket increased by a factor of 2. The calibration equation obtained at 70°C was $\Delta A = (8.67 \pm 0.53) \times 10^{-3}[\text{HCHO}, \mu\text{M}] + (2.08 \pm 2.96) \times 10^{-3}$. The calibration obtained at 25°C was only $\Delta A = (4.55 \pm 0.10) \times 10^{-3}[\text{HCHO}, \mu\text{M}] + (0.22 \pm 0.55) \times 10^{-3}$. The highly reproducible signals (%RSD = 0.7) obtained over an extended operating period of 36 h indicated that the heating jacket was efficient. Therefore, this electrical heating jacket was employed for all further experiments, operating at 70°C.

Design of a procedure for multiple processing

With the normal procedure, the analysis time for one sample is 7.5 min, which includes the time for sample introduction, premixing and pre-heating of the reagents (R_1 and R_2) in the SP_2 syringe, and for detection of the signal at the spectrometer. The introduction of a following sample does not start until the cycle

Table 1 Procedure selected for multiple processing of the flow system in Fig. 1 for the determination of HCHO in food

Step	Motion of pump	Duration/s	Position of analytical zone
1 (load)	850 µl of carrier and 350 µl of sample 1 (S1) are drawn from the sample tube into SP_1 100 µl of reagents (R_1/R_2) are alternately drawn into the SP_2 syringe until total volume reaches 1200 µl	30	
2 (inject)	SP_1 and SP_2 push all solutions from the two syringes to mix and stop at RC	56	
3 (load)	850 µl of carrier and 350 µl of sample 2 (S2) are drawn into SP_1 100 µl of reagents (R_1/R_2) are alternately drawn into SP_2 until total volume reaches 1200 µl	30	
4 (inject)	SP_1 and SP_2 push all solutions from the syringes to mix and stop at RC	56	
5 (load)	850 µl of carrier and 350 µl of sample 3 (S3) are drawn into SP_1 100 µl of reagents (R_1/R_2) are alternately drawn into SP_2 until total volume reaches 1200 µl	30	
6 (inject)	SP_1 and SP_2 push all solutions from the syringes to mix and stop at RC	56	

R_1, 0.04 M 2,4-pentanedione; R_2, 2.0 M ammonium acetate. From step 6 onwards, the flow system handles 3 samples at a time, leading to the throughput of 51 samples h^{-1}.

of the previous sample has been completed. The sample throughput for this normal procedure is therefore low (8 samples h^{-1}). In order to improve the sample throughput, we designed a new operating procedure for the flow system. Table 1 gives this new procedure, which now has a considerably higher sample throughput.

With this new procedure (Table 1), as the first sample (S1) is being heated in the reaction coil RC in step 3, the next sample (S2) is introduced into the holding coil, HC. During this time, pre-mixing and pre-heating of reagents R_1 and R_2 is being processed inside the heated syringe SP_2. Following this step, S2 and the heated reagents are pushed by both syringe pumps into the reaction coil. When the syringe pistons are moved upward, the head of the S1-zone is therefore pushed further into the detector. At the end of step 4, we see the first part of the signal of S1 (Fig. 2a). In step 5, S1 is stationary, and therefore the signal level of S1 stays constant. In step 5, the third sample (S3) is now introduced into the coil, HC. Again, the pre-mixing and pre-heating of R_1 and R_2 occur inside the SP_2 heated syringe. In step 6, S3 and the mixed reagents are pushed by SP_1 and SP_2, to replace S2 in the reaction coil. In step 6, the entire zone of S_1 is now driven through the detection cell to waste giving the complete signal of S1, as shown in Fig. 2a, whereas sample S2 is just entering the flow cell of the detector. After the complete profiling of S1, the system then handles 3 samples at a time, by repeating steps 1 to 6. In this second cycle, the profile of S2 appears in step 1, and finishes at the end of step 2 (Fig. 2b). The profile of S3 appears in steps 3 and 4 (Fig. 2c), and so on.

System optimization

The effect of the flow rates for lines AC and BC of the system (Fig. 1) were examined. It was observed that the signal

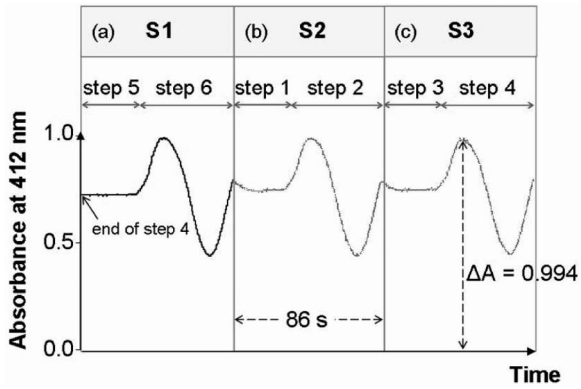


Fig. 2 Signal profiles obtained from the consecutive introduction of the liquid standard of HCHO (100 μM), representing the ability of the flow system in multiple processing of three samples (S1, S2 and S3) all at the same time, under the operating procedure given in Table 1. The baseline is set at zero absorbance.

decreased as the flow rate was increased from 0.1 to 0.8 ml min^{-1}. As a compromise between the signal amplitude and the speed of analysis, 0.6 ml min^{-1} was chosen.

Various sample volumes ranging from 50 - 1200 μl were investigated. The signal increased with increasing sample volume, reaching a plateau for a volume greater than 500 μl. In this work, we selected 350 μl as the sample volume instead of 500 μl to reduce the processing time of a sample.

The effect of various concentrations of 2,4-pentanedione was also studied. The signal increased sharply from 0.02 to 0.03 M, but stayed more or less constant between 0.03 and 0.05 M. Therefore, 0.04 M of 2,4-pentanedione was chosen. Ammonium acetate concentrations from 1 to 4 M were investigated. It was found to have only a little effect on the signal at concentrations above 2 M. For this work, 2 M of ammonium acetate was chosen.

Analytical features

Using the procedure given in Table 1 and the optimum conditions, the plot between the peak height (ΔA) and the concentration of HCHO, over the range of 10 to 100 μM, was linear ($\Delta A = (9.85 \pm 1.34) \times 10^{-3}$[HCHO, μM] – $(7.35 \pm 1.20) \times 10^{-3}$, $r^2 = 0.999$). Ten replicate injections of 40 μM HCHO were carried out to obtain the system's precision of 0.9% (RSD). A concentration of 1 μM was shown experimentally to be the limit of detection, LOD (3SD of blank signal, $n = 7$), of a sample extract. This translates to a LOD of 6.0×10^{-2} mg kg^{-1} for a solid sample. For our method, the limit of quantitation, LOQ (5SD of blank signal), was found to be 1.0×10^{-1} mg kg^{-1}. The LOQ of our method meets the acceptable residue levels in vegetable (63 mg kg^{-1})[25] and seafood (10 mg kg^{-1} for crustaceans and 60 mg kg^{-1} for *Gadidae* fish).[4]

This developed flow system has a special feature of multiple processing of samples and reagents. With this capability of handling multiple tasks at the same time, the flow system has a high sample throughput of up to 51 food samples h^{-1}.

The signal profiles obtained from our system (Fig. 2) are different from what is normally seen with general flow systems. In Fig. 2c, we show the measurement of a signal obtained from 100 μM HCHO ($\Delta A = 0.994$). The zero absorbance reading of the detector was set with water.

Table 2 Results of formaldehyde analysis in re-hydrated dry squid obtained from our flow method and from the PRA method

Sample of re-hydrated dry squid	HCHO/mg kg^{-1} ($n = 3$)	
	Our method	PRA method[26]
Sq1	12.37 ± 0.02	12.67 ± 0.03
Sq2	11.81 ± 0.03	10.51 ± 0.05
Sq3	4.51 ± 0.04	4.24 ± 0.00
Sq4	4.10 ± 0.10	4.30 ± 0.00
Sq5	0.26 ± 0.01	0.16 ± 0.00
Sq6	0.31 ± 0.02	0.24 ± 0.00

Applications in food samples

The current method has been applied to local food samples. A total of 18 vegetable samples were analyzed. Samples were selected from cabbages, string beans, kales, together with three kinds of mushroom, shitake, Jew's ear and straw mushroom commonly used in Thai and Chinese cooking. The results showed that the samples were not contaminated with HCHO.

Analyses carried out by our method for re-hydrated dry squid (Table 2) showed that two samples (Sq1 and Sq2) contained high levels of HCHO. However, there is insufficient information to conclude whether the levels found in these two samples are natural levels, or from an exogenous source. For Sq3 and Sq4, the levels were more or less comparable with the normal levels found in cuttlefish (2.91 to 3.27 mg kg^{-1}).[4] The levels of HCHO in Sq5 and Sq6 were at the detectable limit of the developed method.

The conventional PRA method[26] was also employed for validation purpose. The results obtained by the two methods (Table 2) were compared using the Paired *t*-test.[27] There was no significant difference ($t_{observe} = 1.188$, $t_{critical} = 2.776$, $P = 0.05$) in the sets of results obtained from the current method and from the conventional method. This equality between the two methods demonstrates that the newly developed flow method is suitable for the analysis of HCHO contamination in food samples.

Recovery studies were carried out using another set of squid samples ($n = 8$) containing low levels of HCHO, from 0.26 to 0.88 mg kg^{-1}. Analytical recoveries from 93 to 112% were obtained from these squid samples. Recoveries carried out on vegetables' and mushrooms' extracts varied from 92 to 113% (18 samples). These good recovery values indicate that sample matrices did not interfere with our method.

Conclusions

We developed a new operating procedure for multiple tasking of a hybrid flow system for the determination of HCHO. The system allows much faster throughput than previous systems presented for food samples.[19,21,28,29] The employment of the heating device significantly improved the sensitivity of the spectrometric detection by the Hantzsch reaction. The method has sufficient sensitivity to detect HCHO in food, especially in re-hydrated dry squid. Although Thailand has not yet set a maximum limit for the level of HCHO in food, our flow system will be a useful tool for screening the misuse of HCHO.

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