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# Analysis of Acrylamide in a Complex Matrix of Polyacrylamide Solutions Treated by Heat and Ultraviolet Light

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A recently developed headspace/solid-phase microextraction/gas chromatograph (GC) equipped with a nitrogen−phosphorus detector (NPD) (HS/SPME/GC/NPD) method was used to analyze acrylamide formed in an aqueous polyacrylamide solution (25%) treated by heat or photo-irradiation. Original polyacrylamide contained 0.43 ± 0.11 μg/mL of acrylamide. When polyacrylamide solution was heated at 70 °C for 16 h with 0.5% potassium persulfate, the amount of acrylamide increased to 1.02 ± 0.11 μg/mL. When polyacrylamide solution was irradiated by UV ($\lambda$ = 300 nm) for 16 h with 0.05% 2-anthraquinone sulfate sodium salt, the amount of acrylamide increased to 1.14 ± 0.54 μg/mL. Polyacrylamide has been used in cosmetic formulations. The present study, therefore, suggests that there is another route of acrylamide exposure to humans in addition to foods and beverages.

**KEYWORDS: Acrylamide; cosmetics; gas chromatography; headspace; polyacrylamide; solid-phase microextraction**

## INTRODUCTION

Acrylamide is an important industrial chemical and has been known for many years to be present in drinking water and tobacco smoke (*1*). The discovery of significant levels of acrylamide in heat-processed starch-based foods, such as potato chips and French fries, in April 2002 (*2*), triggered intensive studies to determine acrylamide in foods. Moreover, the discovery of acrylamide in foods has prompted worldwide attention because it has been considered a probable human carcinogen, a neurotoxicant, and a genotoxicant (*3, 4*).

Polyacrylamide has been used for various purposes, including the removal of suspended solids from industrial wastewater, as a soil conditioner, grouting agent, surfactant for herbicide mixtures, and stationary phase for laboratory separations, and in cosmetic formulations. Polyacrylamide is formulated in over 100 cosmetic products as a stabilizer, foam builder, binder, film former, antistatic agent, and hair fixative at concentrations ranging from 0.05 to 2.8% (*5*). Residual acrylamide in polyacrylamide is reported to range from 0.02 to 0.2 μg/mg in various cosmetics (*6*). However, polyacrylamide has been known to degrade into acrylamide monomer during thermal treatment or during UV exposure in the presence or absence of radical initiators under some artificial environmental conditions (*7*). Unlike the acrylamide monomer, which readily penetrates the skin, polyacrylamide polymers do not penetrate dermally because of their large sizes and thus are generally nontoxic (*8*). It is the degradation product, acrylamide, that is a toxicant, which produces axonopathy by transection of neurons (*9*). Therefore, to assess the adverse effects of acrylamide, direct exposure through the skin as well as its ingestion through foods should be considered.

Many methods had been developed for analyzing acrylamide in various matrices, including water (*10*), tomato fruit (*11*), and mushroom (*12*), before acrylamide was found in cooked foods. Currently, the most widely used analytical method for acrylamide is liquid chromatography/tandem mass spectrometry (LC/MS/MS) (*13*) because it is specific and sensitive for acrylamide; moreover, it does not require tedious sample preparation (*14*). However, if acrylamide monomers were not resolved well from other degradation products of polyacrylamide, such as acrylamide dimer and trimer, on a chromatographic column interfaced to a MS, acrylamide monomer samples containing polyacrylamide give a high background noise based on the fragments at *m/z* 72 and 58 because of the similarity of their chemical structures, resulting in low selectivity on monomer. Therefore, GC/MS may give a higher selectivity because GC has a better resolution for acrylamide monomers than HPLC. However, sample preparation of acrylamide from a mixture of monomer and polymers for GC is not easy because both the monomer and polymers of acrylamide are highly soluble in water.

Recently, a new method to collect acrylamide from aqueous solutions using headspace solid-phase microextraction (HS/SPME) for a gas chromatograph with a nitrogen−phosphorus detector (GC/NPD) was developed (*15*). In the present study, this recently developed HS/SPME/GC/NPD method was used

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to analyze acrylamide formed from polyacrylamide by heat and light degradation.

## MATERIALS AND METHODS

**Materials.** Polyacrylamide (typical MW 10000, 50% in water), acrylamide, 2-methylacrylamide, potassium persulfate (PPS), and 2-anthraquinone sulfate sodium salt (AQS) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). HPLC grade water was purchased from Fisher Co. (Pittsburgh, PA). Carboxen/polydimethylsiloxane fiber (CAR/PDMS, 65 $\mu$m thickness) and a fiber assembly holder were purchased from Supelco, Inc. (Bellefonte, PA).

**Thermal Treatment of Polyacrylamide.** Two milliliters of polyacrylamide solution (25% in water) with PPS (0.0, 0.5, or 1.0%, w/v) was placed in an 8 mL scintillation vial. The vial was capped with a Teflon gasket and placed in a thermally controlled oven. The mixture was heated at 70 °C for 16 h. After thermal treatment, the vial was cooled to room temperature and kept at 5 °C until acrylamide analysis. The original polyacrylamide solution was analyzed for acrylamide as a blank sample. The polyacrylamide solution with no PPS was used as a control sample.

**Photo-irradiation of Polyacrylamide.** Five milliliters of a polyacrylamide solution (25% in water) with AQS (0.0, 0.05, or 0.2%, w/v) was put in a glass tube. The glass tube was capped with a Teflon gasket and placed in a photochemical reactor (The Southern New England Ultraviolet Co., Hamden, CT). The mixture was irradiated by UV lamp ($\lambda = 300$ nm) at $0.68 \pm 0.2$ mW/cm$^2$ ($n = 6$) for 16 and 64 h at room temperature. After UV exposure, the tube was covered with aluminum foil and kept at 5 °C until acrylamide analysis.

**Quantitative Analysis of Acrylamide Formed from Polyacrylamide.** Quantitative analysis of acrylamide was performed using a previously reported HS/SPME/GC/NPD method (*15*). Two milliliters of polyacrylamide solution (25% in water) and 100 $\mu$L of an internal standard solution (2-methylacrylamide, 1 mg/mL) were mixed immediately before HS/SPME. A sample solution (1 mL) containing 50 $\mu$g/mL 2-methylacrylamide was placed in a 4 mL glass vial with a Teflon/silicone rubber septum. The tube was immersed in a thermostatic water bath (Isotemp water bath, model 2LS-M, Fisher Scientific, Pittsburgh, PA) set at 60 °C for 10 min. A SPME holder equipped with a CAR/PDMS fiber was inserted into the headspace above the reaction solution, and acrylamide was extracted for 60 min at 60 °C. The SPME fiber was immediately transferred to the injector of the GC and desorbed at 200 °C for 10 min. Acrylamide was analyzed by a GC equipped with an NPD in triplicates.

The acrylamide concentration was determined using the GC peak area ratio of acrylamide and 2-methylacrylamide spiked as an internal standard. A calibration curve was prepared using a series of acrylamide standard solutions prepared in a 25% polyacrylamide solution, with concentrations ranging from 0.1 to 2.0 $\mu$g/mL. The correlation coefficient showed good linearity in the range under consideration ($r^2 > 0.990$). The detection limit was calculated as 3 times the standard deviation. The quantification limit was calculated as 10 times the standard deviation. Identification of acrylamide in a sample was confirmed by a GC/mass spectrometer (MS).

**Recovery Efficiency of Acrylamide from Polyacrylamide Solution.** For the recovery test, 1 mL of aqueous polyacrylamide solution (25%) was spiked with 100 $\mu$L each of an aqueous acrylamide solution (10 $\mu$g/mL) and an aqueous 2-methylacrylamide solution (50 $\mu$g/mL) in a 4 mL glass vial with a Teflon/silicone rubber septum. Therefore, the level of acrylamide in a testing solution was approximately 1 ppm. The headspace of the vial was extracted with an HS/SPME and subsequently analyzed by a GC/NPD in triplicate. Recovery was calculated after blank correction.

**Instrumentation.** An Agilent model 6890 GC equipped with a 30 m $\times$ 0.25 mm i.d. ($d_f = 0.25$ $\mu$m) ZB-WAX fused silica capillary column and an NPD was used. An SPME injection sleeve (Supelco, Inc.) was used for the glass insert of the injector. The injector temperature was 250 °C at splitless mode, and the detector temperature was 250 °C. The column was held at 40 °C for 2 min, then programmed to 180 at 4 °C/min, and held for 8 min. The linear helium carrier gas flow rate was 30 cm/s. The nitrogen makeup gas flow rate was 10.0 mL/min; the flow rates of hydrogen and air were 3.0 and 60.0 mL/min, respectively. Under these conditions acrylamide eluted at 31.8 min. A Hewlett-Packard model 5890 series II GC interfaced to an HP 5971 mass spectrometer equipped with a 60 m $\times$ 0.25 mm i.d. DB-Wax fused silica capillary column ($d_f = 0.25$ $\mu$m) was used to identify acrylamide. The injector temperature was 250 °C at splitless mode. The column was held at 40 °C for 10 min, then programmed to 200 at 5 °C/min, and held for 10 min. The linear helium carrier gas flow rate was 28 cm/s. Under these conditions acrylamide eluted at 40.5 min. The mass spectra were obtained by electron impact ionization at 70 eV at an ion source temperature of 250 °C.

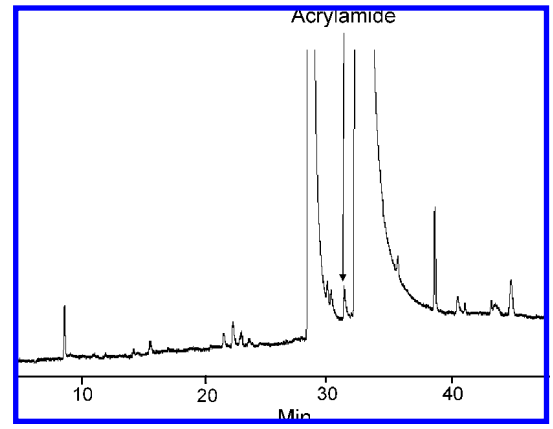


**Figure 1.** Typical gas chromatogram of a headspace sample collected with a CAR/PDMS fiber from a polyacrylamide solution heated at 70 °C for 16 h.

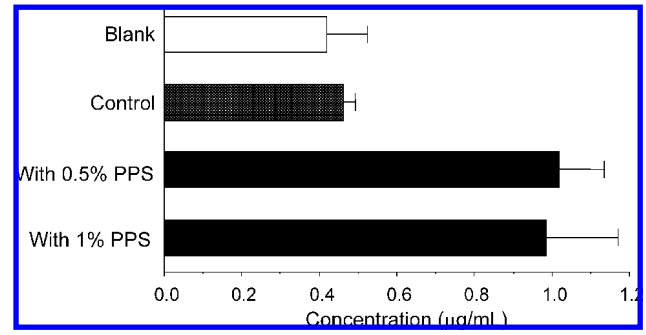


**Figure 2.** Amounts of acrylamide found in thermally treated polyacrylamide solutions.

## RESULTS AND DISCUSSION

**Figure 1** shows a typical gas chromatogram of headspace sample obtained from a polyacrylamide solution heated at 70 °C for 16 h. CAR/PDMS fiber was chosen because it gave the highest efficiency of extraction among various fibers tested previously (*15*). The recovery efficiency for acrylamide using the HS/SPME/GC/NPD method was $99.8 \pm 2.4$%. The values are mean $\pm$ standard deviation ($n = 3$). The detection limit and quantification limit were 0.06 and 0.19 $\mu$g/mL, respectively.

**Figure 2** shows the amount of acrylamide found in thermally treated polyacrylamide solutions along with their blank and control samples. Original polyacrylamide contained $0.43 \pm 0.11$ $\mu$g/mL (blank) of acrylamide monomer. When polyacrylamide was heated at 70 °C for 16 h without PPS (control), the amount of acrylamide in polyacrylamide increased to $0.46 \pm 0.03$ $\mu$g/mL. When 0.5 or 1.0% PPS was added to a polyacrylamide solution, the amount of acrylamide increased to $1.02 \pm 0.11$ or $0.98 \pm 0.19$ $\mu$g/mL, respectively, after thermal treatment. The results indicate that acrylamide was formed by thermal degradation of polyacrylamide and that radical initiator (PPS) accelerated the acrylamide formation.

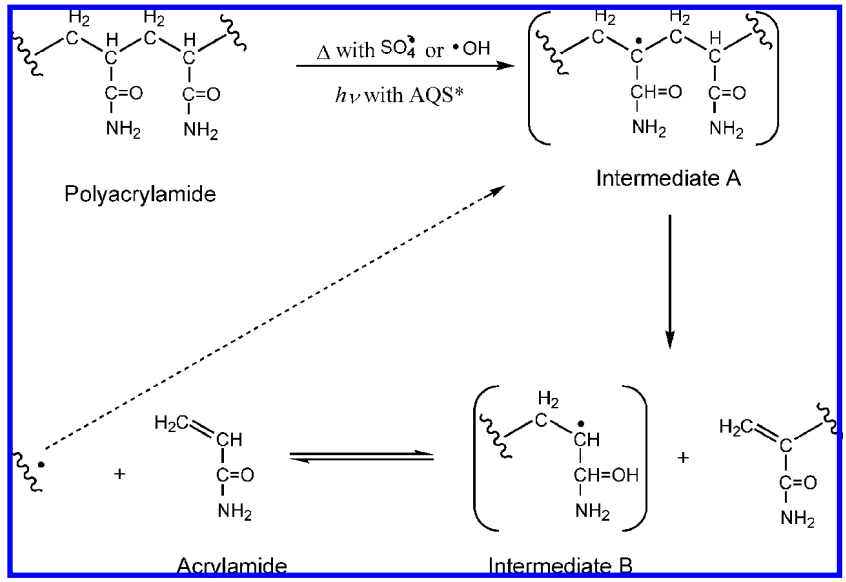


**Figure 3.** Hypothesized formation mechanisms of acrylamide from polyacrylamide.

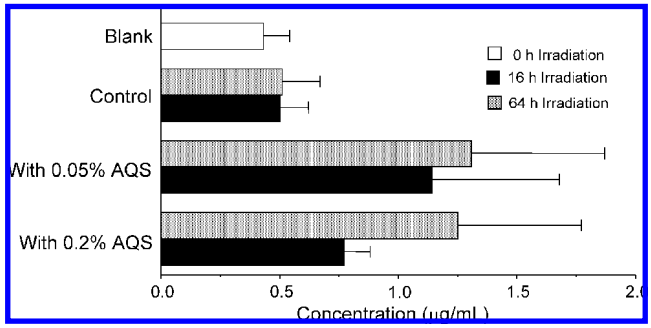


**Figure 4.** Amounts of acrylamide formed from polyacrylamide upon UV irradiation.

Polyacrylamide has been known to degrade into acrylamide monomer by thermal treatment or UV exposure in the presence or absence of radical initiators (*16*). Potassium persulfate was used as a radical initiator for a study on the polyacrylamide degradation (*17*). AQS has been used for a surface modification of polymers by UV irradiation (*7*). The mechanism of polyacrylamide degradation was attributed to hydrogen atom abstraction from α-carbon by radical species. Acrylamide may be formed by radical degradation of polyacrylamide.

**Figure 3** shows the hypothesized formation mechanisms of acrylamide from polyacrylamide with these radical initiators in aqueous solution. PPS generates sulfate radicals and hydroxyl radicals via thermal decomposition (*18*). The persulfate ion from PPS forms sulfate ion radical, which subsequently reacts with a water molecule to yield a hydroxyl radical in an aqueous solution (*19*). Obvious degradation of polyacrylamide was observed when it was treated by a hydroxyl radical generated from Fenton's reagent in an aqueous solution (*20*). In the present study, it is hypothesized that acrylamide formed from one of the radical intermediates (intermediate B in **Figure 3**), which were previously reported (*21*), formed from polyacrylamide via radical degradation in an aqueous solution.

**Figure 4** shows the amount of acrylamide formed from polyacrylamide upon UV irradiation. When polyacrylamide was irradiated without AQS, the amount of acrylamide in polyacrylamide increased from 0.43 ± 0.11 μg/mL (blank) to 0.50 ± 0.12 μg/mL (control). When 0.05% AQS was added to a polyacrylamide solution, the amount of acrylamide increased from 0.50 ± 0.12 to 1.14 ± 0.54 μg/mL and from 0.51 ± 0.16 to 1.31 ± 0.56 μg/mL after UV irradiation for 16 and 64 h, respectively. When 0.2% AQS was added to a polyacrylamide solution, the amount of acrylamide increased from 0.50 ± 0.12 to 0.77 ± 0.11 μg/mL and from 0.51 ± 0.16 to 1.25 ± 0.52 μg/mL after UV irradiation for 16 and 64 h, respectively.

The results indicate that acrylamide was formed by photoirradiation and that a photosensitizer accelerated the acrylamide formation. AQS yields n−π* triplet state by UV irradiation. The excited AQS sensitized to form a radical-bearing polymer (intermediate A in **Figure 3**), and then the degradation to yield acrylamide may occur in the same way as thermal degradation as shown in **Figure 3**. Some water-soluble polymers including polyacrylamide were degraded significantly by ozonation under UV irradiation (*22*). However, degradation products were not reported in this study.

Other studies also indicated that polyacrylamide produced acrylamide monomer under some conditions including artificial environment (*16*) and thermal/UV irradiation (*23*). The wavelength used (300 nm) was a typical cutoff wavelength of Pyrex, and it is commonly used for artificial UV irradiation studies by sunlight. Therefore, the conditions used in the present study are not too far from the actual conditions to which cosmetic products are exposed. It is a general practice to add polyacrylamide into water-based skin care cream, foundation, and other skin care products. Many fragrance chemicals, which contain some photosensitizers such as benzophenone, benzaldehyde, and acetophenone, are also commonly formulated with those skin care products. Therefore, acrylamide may form from skin care products after they are applied on human skin and exposed to sunlight.

It has been reported that residual acrylamide monomer is likely an impurity in most polyacrylamide formulations in cosmetics as mentioned above. Therefore, it is estimated that the use of polyacrylamide in cosmetics may result in a heavy user of the cosmetics having a potential daily exposure to 67 μg of acrylamide monomer (*24*). The Cosmetic Ingredient Review (CIR) Expert Panel concluded that it was appropriate to limit acrylamide levels to 5 ppm in cosmetic formulations (*5*).

It should be noted that these assessments have been made on the basis of the residual acrylamide levels in cosmetic products before they are applied to the skin. However, the present study showed that acrylamide monomer was formed

from polyacrylamide by heat and photoirradiation even though the concentrations of polyacrylamide used in the present study are much higher than those used in cosmetics. Therefore, further investigation on acrylamide risk assessment associated with exposure routes, other than food, including skin, is in order.

One of the greatest drawbacks of GC is that a sample has to be water free. Therefore, tedious sample preparation steps, such as repeated extraction with an organic solvent, cleanup with column chromatography, removal of solvents, and dissolving of the final sample in an appropriate organic solvent, are often required for GC analysis. The present study demonstrated that our recently developed HS/SPME/GC/NPD method, the sample preparation steps of which are considerably simpler, could be applied to analyze acrylamide present in complex matrices such as foods and cosmetics.

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